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EPITHELIAL CONDUCTION AND THE CONTROL OF CRUMPLING BEHAVIOR IN *POLYORCHIS PENICILLATUS*.

by

© MICHAEL G. KING

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

DEPARTMENT OF ZOOLOGY

EDMONTON, ALBERTA

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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled Epithelial conduction and the control of crumpling behavior in *Polyorchis penicillatus*.

submitted by Michael G. King

in partial fulfilment of the requirement for the degree of Master of Science.

Supervisor

Date Aug. 9, 1977

DEDICATION

To my wife and daughters for their gracious and patient acceptance of time lost and to my parents for their inspirational support which defies geographical separation.

ABSTRACT

Two aspects of the neurobiology of the hydrozoan jellyfish *Polyorchis penicillatus* were investigated with electrophysiological and ultrastructural techniques.

Extracellular recordings from the endoderm and the exumbrellar ectoderm demonstrate that these epithelia have the ability to conduct action potenials. Intracellular recordings from the endoderm verify that this excitability is a property of the epithelial cells. Current pulses were shown to pass between intracellular electrodes in two separated endodermal cells, demonstrating that low-resistance intercellular pathways electrically couple adjacent cells.

The probable structural correlate of epithelial conduction was eludicated using lanthanum impregnation and freeze-fracture replication electronmicroscopy. Within the endodermal canal, arrays of septate junctions are prevalent around the canal lumen and gap-junctions are concentrated along the canal periphery. Septate junctions have a regular intercellular space of 13-14nm with 7.5nm wide septa displaying periodicities of 14-16nm. In freeze-fracture replicas these septa appear as a series of EF particle rows with complementary PF grooves. Gap-junctions have intercellular spaces of 4.5nm in conventionally stained material and show 6-7nm bridges, with a periodicity of 10-12nm, connecting apposed membranes in lanthanum impregnated material. In freeze-fracture replicas, gap-junctions occur as aggregates of EF particles with diameters of 9.5-11nm and a periodicity of 11-12.5nm. It is suggested that both gap and septate junctions are involved in impulse propagation, the former providing

intercellular current pathways and the latter limiting the shunting of current flow between cells by sealing the extracellular space from the surrounding gut saline.

Crumpling is a protective response shown by many hydromedusae involving simultaneous contraction of the four subumbrellar radial muscles concomitant with sphincter muscle contraction and retraction of the tentacles and manubrium. From the observation that stimulation of the exumbrellar ectoderm elicits crumpling and endodermal impulses concurrently, it was previously hypothesized that subumbrellar endoderm directly excites the ectodermal radial muscles.

To determine whether there is a direct epithelial excitation of radial muscle in *Polyorchis*, extracellular recordings were made from radial muscle and endoderm. During crumpling a complex potential is recorded from the radial muscle due to the simultaneous activity of the radial muscle and underlying endoderm. By various manipulations this compound potential was separated into its component radial muscle potential (RMP) and endodermal canal pulse (ECP). By comparing the response latencies of RMPs recorded at various locations along the muscle it was determined that no direct excitation is transmitted from endoderm to radial muscle. It was also found that epithelial initiation of radial muscle response occurs only at the marginal and apical ends of a muscle. Electron microscopy has shown that there are synapses between radial nerve and radial muscle throughout the radius and between marginal nerves and radial muscle. It is suggested that nerves are integral to the crumpling pathway serving to transmit excitation from epithelia to radial muscle. This system may serve as an important model for epithelial-nervous interactions.

ACKNOWLEDGEMENTS

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For the electron microscopy, I express my gratitude to Dr. S.K. Malhotra for generously providing research facilities and to the EM staff for their upkeep. I would also like to thank Dr. Mike Tu for teaching me the techniques of [redacted] tore replication and Dr. J.P. Tewari for its interpretation. In addition, [redacted] thanks must be extended to Dr. E. Huebner for technical advice regarding lanthanum-impregnation and to Dr. R. Burke and E. Koss for useful tips on general EM techniques.

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INTRODUCTION

Cnidarian nervous systems have attracted a number of physiologists and anatomists over the past century (reviewed by Josephson, 1974; Chapman, 1974), primarily on the presumption that the lower evolutionary status of these animals should facilitate analysis of functional and structural mechanisms underlying behavior and perhaps provide a prototype for considerations of the origin of higher nervous systems. It is now apparent that the behavioral patterns and possible conduction pathways of cnidarians are simplified relative to other animals, and therefore amenable to research, although their degree of complexity and variability makes their usefulness as a representative ancestor of higher nervous systems uncertain.

Within this phylum neurophysiological analysis has primarily concerned anthozoans, especially sea anemones, and the aberrant *Hydra*. Most electrophysiological investigations of hydrozoan jellyfish have entailed a description of the impulse types correlated with observed behavior in anthomedusae (Passano, 1965; Mackie *et al.*, 1967; Passano *et al.*, 1967; Mackie and Passano, 1968; Ohtsu and Yoshida, 1973; Mackie, 1975; Spencer, 1978), a limnomedusan (Spencer, 1975) and siphonophores (Mackie, 1965, 1976a; Bassot *et al.*, 1978) or a verification of cellular excitability and interactions with intracellular electrodes (Spencer, 1971; Mackie, 1976a, b; Anderson and Mackie, 1977; Spencer, 1978). There is, however, a scarcity of studies establishing the exact conduction pathways followed in eliciting hydromedusan behavior.

The notion that Cnidarians possess general conduction pathways supplementary to nerves is not a recent innovation. Chun (1897) proposed that the myoepithelial layer as well as the exumbrellar ectoderm of siphonophores may be conductile. Although it was subsequently suggested that epithelial conduction plays a role in the behavior of *Hydra* (Hadzi, 1909), a cerianthid sea anemone (Horridge, 1958) and a hydroid (Josephson, 1965), it was not until the histological proof of a nerve-free epithelium which propagates impulses (Mackie, 1965) that the phenomenon of epithelial conduction (in any organism) was accepted. Although there is indirect evidence that *Hyrda* may have excitable epithelia (Campbell *et al.*, 1976; Kass-Simon and Diesl, 1977) excitability has been confirmed with intracellular recording of epithelial action potentials only in a siphonophore (Mackie, 1976b) and two anthomedusae (Spencer, 1978; Josephson and Schwab, in preparation).

It is now established that impulse-propagating epithelia are not restricted to hydromedusae. They have also been described in frog tadpoles (Roberts, 1969; Roberts and Stirling, 1971), larval (Mackie and Bone, 1976) and adult urochordates (Bone and Mackie, 1975; Mackie and Bone, 1977), mammalian endocrine gland (Matthews and Saffran, 1973; Matthews and Sakamoto, 1975), gastropod exocrine gland (Kater *et al.*, 1978b) and polychaete elytra (Herrera, 1979). Although the physiology of these conducting epithelia has been studied in depth, there is a lack of detailed ultrastructural evidence for specialized intercellular junctions that can be associated with this excitability. Close membrane appositions

have been reported in some of these epithelia (Roberts and Stirling, 1971; Joseph *et al.*, 1973; Bone and Mackie, 1975; Mackie and Singla, 1975; Mackie, 1976b; Mackie and Bone, 1976; Bassot *et al.*, 1978). However, only Orci, Unger and Renold (1973) have contributed indisputable evidence for membrane junctions likely to be correlated with excitability in an epithelium (Matthews and Sakamoto, 1975).

Substantial evidence for intercellular contacts probably associated with impulse conduction has been provided in vertebrate cardiac (Revel and Karnovsky, 1967; McNutt and Weinstein, 1970) and smooth (Friend and Gilula, 1972) muscle, vertebrate central nervous tissue (Brightman and Reese, 1969; Zampighi and Robertson, 1973; Bennett *et al.*, 1978) and crustacean nerve cord (Pappas *et al.*, 1971; Peracchia, 1973). It is generally accepted that gap-junctions are the sites of impulse transfer between cells within these tissues. By extension, it is assumed that gap-junctions provide the mechanism for conduction of actions potentials in excitable epithelia. Gap-junctions have also been implicated in the maintenance of electrical and nutritional coupling in a variety of inexcitable epithelia (reviewed by Sheridan, 1974; Staehelin, 1974; Gilula, 1977; Pitts, 1977). The electrical coupling of these latter epithelia is not associated with impulse propagation and is of significance only in demonstrating low-resistance intercellular connections presumably through structural bridges at the gap-junctions (reviewed by Loewenstein, 1977).

The basic function of epithelial conduction is to furnish receptive fields and pathways for the mediation of an escape or

protective response. This involves either a protective closure (Mackie, 1965; Mackie and Passano, 1968) or an activation (Mackie, 1964; Roberts, 1971; Bone and Mackie, 1975; Mackie and Bone, 1977) or inhibition (Mackie and Bone, 1976) of locomotion. Alternatively, epithelial excitability can be correlated with glandular secretion (Matthews and Saffran, 1973; Matthews and Sakamoto, 1975; Mackie, 1976; Kater *et al.*, 1978b) or bioluminescence (Bassot *et al.*, 1978; Herrera, 1979).

In many hydromedusae the exumbrellar ectoderm and entire endoderm is excitable and epithelial conduction is associated with the activation of "crumpling" behavior (Mackie and Passano, 1968). This response involves a simultaneous contraction of the subumbrellar radial muscles and marginal sphincter muscle and retraction of the tentacles, manubrium and gonads. This results in an involution of the margin, reducing the opening of the bell and enclosing the delicate marginal tissues within the subumbrellar cavity.

This behavior was described by Romanes (1876) who termed it a "spasm" and was rediscovered by Hyman (1940) who first referred to is as "crumpling". It was not, however, until Mackie, Passano and Pavans de Ceccatty (1967) that the prime effector of the response, the subumbrellar, ectodermal radial muscles, was established. This clarified the basis of the response, correcting the earlier interpretation (Romanes, 1876) that swimming and crumpling result from contraction of the subumbrellar circular muscle. Concurrent with the description of excitable epithelia in hydromedusae (Mackie *et al.*, 1967; Mackie and Passano, 1968), it was proposed

that excitation passes from exumbrellar ectoderm to endoderm at the margin and then back to ectoderm in the subumbrella to activate the radial muscles in *Sarsia* and *Euphysa*. The presence of epithelial bridges between ectoderm and endoderm at the margin and perradius (Mackie and Passano, 1968; Mackie and Singla, 1975; Spencer, 1979) seems to correlate with this hypothesis. The epithelial pathway of crumpling has also been proposed to occur in *Stomotoca* (Mackie, 1975). There is, on the other hand, evidence that epithelial excitation can activate (Mackie and Passano, 1968) or inhibit (Mackie, 1975) nervous activity in anthomedusae. Additionally, asymmetrical excitation of the radial muscles, as occurs during feeding, seems to require nervous control of radial muscle activity.

The purpose of this study was twofold. Firstly, I hoped to correlate the reported epithelial conduction in medusae with definite membrane specializations and particularly to describe structures which could mediate ionic flow between cells (King and Spencer, 1979). Secondly, I wanted to determine the importance of epithelial pathways in activating crumpling behavior in *Polyorchis penicillatus* and to correlate this physiology with ultrastructure.

MATERIALS AND METHODS

Jellyfish were collected from eelgrass beds in Bamfield and Grappler inlets (west coast of Vancouver Island) and held in a flow-through aquarium at about 11°C for a maximum of 5 days. In some instances, jellyfish were transported to the University of Alberta from Vancouver Island and kept in "Instant Ocean" prior to fixation.

Electron Microscopy

For conventional electronmicroscopy a variety of vehicles were used with the standard glutaraldehyde/osmium tetroxide double fixation. The best results were obtained with the following recipes:

1. Fixative - 2% glutaraldehyde in 0.2M sodium cacodylate buffer at pH 7.4 with 10mM $CaCl_2$ and 0.18M NaCl.

   Buffer rinse - 0.2 M sodium cacodylate buffer at pH 7.4 with 10mM $CaCl_2$ and 0.3M NaCl.

   Post-fixative - 1% OsO_4 in 0.2M sodium cacodylate buffer at pH 7.4 with 10mM $CaCl_2$ and 0.28M NaCl.

2. (Modified from Karnovsky, 1967)

   Fixative - 2.5% glutaraldehyde and 2% formaldehyde in 0.1M sodium cacodylate buffer pH 7.4 with 5mM $CaCl_2$ and 0.23M NaCl.

   Buffer rinse - 0.1M sodium cacodylate buffer at pH 7.4 with 5mM $CaCl_2$ and 0.44M NaCl.

   Post-fixative - 1% OsO_4 in 0.1M sodium cacodylate buffer at pH 7.4 with [illegible] $CaCl_2$ and 0.42M NaCl.

3. (Dunlap, 1966)

Fixative - 2.5% glutaraldehyde in 0.2M Millonig's phosphate buffer at pH 7.4 with 0.14M NaCl.

Buffer rinse - 0.2M Millonig's phosphate buffer at pH 7.4 with 0.3M NaCl.

Post-fixative - 1% OsO_4 in 0.1M Millonig's phosphate buffer at pH 7.4 with 0.375M NaCl.

The basic proceedure was to fix the material for 1-1½h at 4°C or room temperature, rinse for 1h with 3-4 changes and post-fix for 2h at 4°C. Dehydration was performed with a graded series of ethanols (30% to absolute) following which the material was treated with propylene oxide for ½h (3 changes) prior to infiltration in a 1:1 mixture of propylene oxide and Araldite for 12h. Treatment with fresh Araldite for 12h at room temperature preceded embedding at 60°C for 36h.

For light-microscopy and orientation puposes, thick (½-1µm) sections were cut with a glass knife and stained with a 1:1 mixture of 1% azure II and 1% methylene blue in 1% sodium borate (Richardson *et al.*, 1960). Thin (gray to gold) sections were cut on a diamond knife, collected on 200 mesh uncoated or 100 mesh parlodian-coated girds and stained for 20 min in 50% ethanol-saturated uranyl acetate followed by 5-8 min in lead citrate (Venable and Coggeshall, 1965).

Lanthanum impregnation (Revel and Karnovsky, 1967) was accomplished by adding lanthanum nitrate to the conventional glutaraldehyde/ formaldehyde fixative and buffer rinse (recipe 2) to give a 2% lanthanum solution followed by a 1h rinse in 0.03N NaOH (Albertini and Anderson, 1974). The tissue was then post-fixed, dehydrated and

embedded as before, Thin sections were either stained with uranyl acetate and lead citrate as before or examined unstained.

Thin sections were viewed in a Philips EM-200 or EM-201 electron microscope.

For freeze-fracture replication, jellyfish were fixed with glutaraldehyde in cacodylate buffer (recipe 1) for 1-2h and after 3 buffer rinses were cryoprotected with 25% glycerol (in recipe 1 rinse) for 1-4h. Material was frozen on gold stubs in Freon-22 and kept in liquid nitrogen prior to fracturing for up to 4 days. The material was fractured under high vacuum in a Balzers BA 360M freeze-etch device. The platinum- and carbon-coated replicas were isolated by digestion of fractured tissue with chromic acid and picked up on 200 mesh naked grids.

Electrophysiology

Extracellular recordings were made with flexible suction electrodes constructed from polyethylene tubing with diameters between 25-100μm. Sea water was used as the electrolyte and was connected to a Tektronix TM 503 preamplifier via platinum or Ag-AgCl wire. Amplified and differentiated signals were fed to a Tektronix 5103N storage oscilloscope and a Gould 220 Brush Recorder for display. 1-2ms square pulses from a Grass SD5 Stimulator were used for tissue stimulation and oscilloscope trace triggering. The use of a WPI 140A Scope Raster/Stepper allowed display of multiple consecutive oscilloscope tracings for analyzing the effect of repetitive stimulation.

Intracellular recordings were made with glass micropipettes filled with 3M KCl and with resistances between 30-60MΩ. Signals were fed into WPI M701 DC Amplifiers and displayed on a Tektronix 5103N storage oscilloscope and a Gould 2400 Brush Recorder. For current injection long (600ms) square pulses from a Grass S44 Stimulator were passed to ground. Difficulties with balancing the intracellular potential via the bridge-circuit of the DC amplifiers made simultaneous voltage measurement and current injection with the same electrode unobtainable.

The principle preparation used was an isolated radius (1 quadrant) of *Polyorchis* consisting of a single radial muscle with attached margin and manubrium. This was pinned to the Sylgard (Dow Corning) base of the recording dish and kept at about 13°C using a glass coil with circulating sea water. In some cases whole animals were used for extracellular recording. Jellyfish were pinned subumbrellar side up without cuts through the margin. The greater activity (compared with the isolated radius) and difficulty of restraining these preparations required the use of slightly larger electrode diameters (75-125μm) and greater suction force. For intracellular recording of the radial canal, the overlying radial muscle was peeled off and the canal was slit along its lumen (Spencer, 1978). Intracellular recording of the endodermal lamella required stripping away a patch of swimming muscle overlying the lamella with a fine pin. Easiest access to these endodermal cells was obtained between lateral branches of the radial canal where the mesogleal layer between subumbrellar ectoderm and endoderm is thinnest.

For the electrophysiology figures, potentials above the base line are positive for intracellular recordings and negative for extracellular recordings. The diagrams which accompany oscilloscope and chart tracings show the locations of the stimulating and recording electrodes using the following symbols: S - stimulating electrode, R1 - recording electrode #1, R2 - recording electrode #2. Unless otherwise stated all figures are oscilloscope tracings.

RESULTS

I. GROSS ANATOMY OF *POLYORCHIS*

The anatomy of *Polyorchis penicillatus* is typical of anthomedusae (Fig. 1). The tall dome-shaped bell consists of an outer exumbrellar and an inner subumbrellar surface. These surfaces join at the velum which is an annular flap projecting inward from the base of the bell or margin. Numerous tentacles, with conspicuous ocelli at their bases, originate at the margin. The manubrium is attached to the apex of the subumbrella by the peduncle.

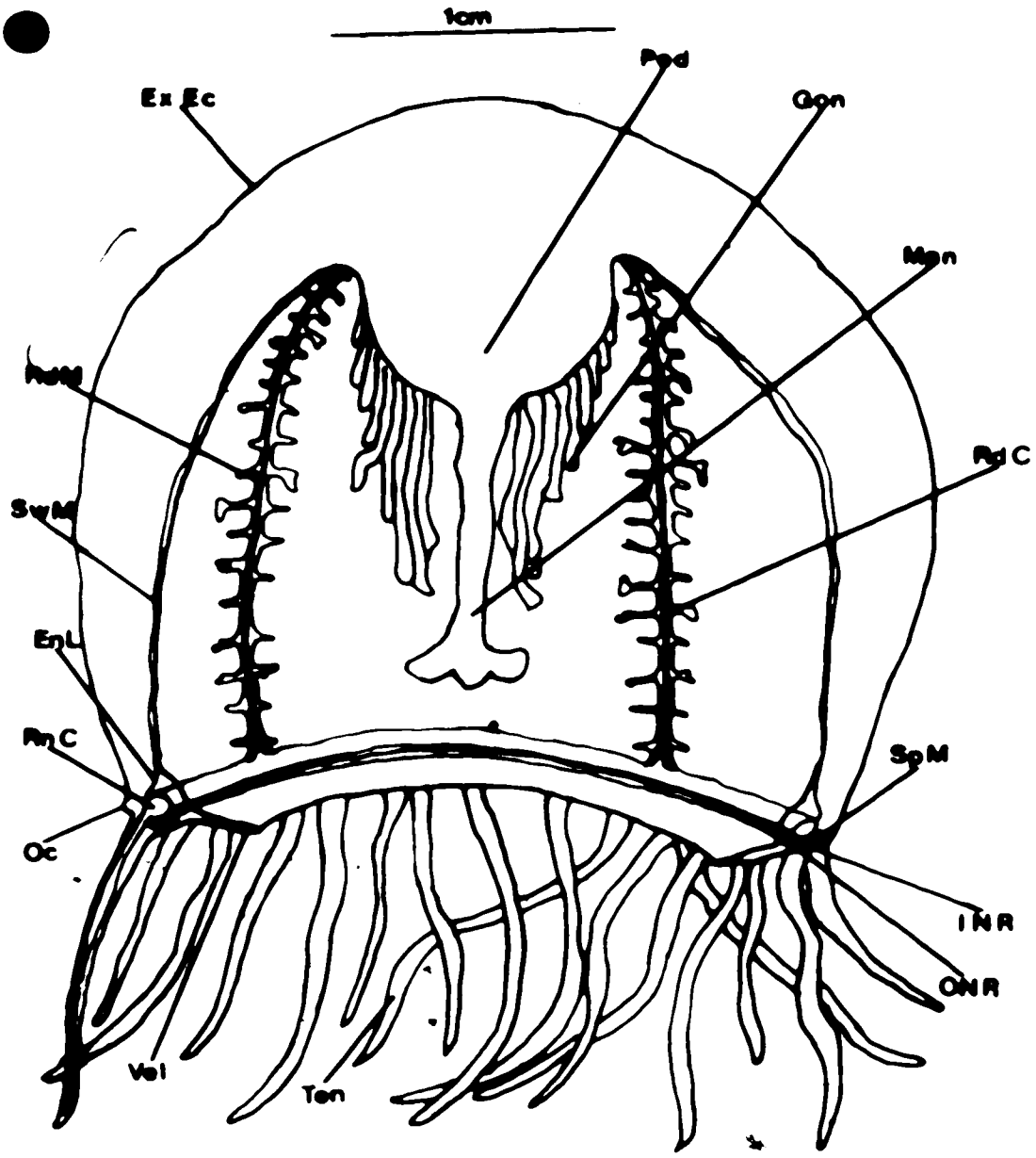
Two major divisions of endoderm are present. Firstly, there is a contiguous canal system consisting of a marginal ring canal with branches into the tentacles, four radial canals (with lateral extensions) which pass to the apex where branches enter the gonads, and the tubular cavity of the manubrium. Secondly, there exists an interradial lamella connected to the radial canals and the ring canal.

The exumbrella consists of a sheet of ectoderm and is separated from the endoderm by a layer of fibrous, acellular mesoglea (bell mesoglea) which is very thick except at the margin. No nerves or myofibrils have been reported in the exumbrella (except at the margin) of any hydromedusan.

It is convenient to distinguish two regions of the subumbrella; the perradius where a radial canal passes from the margin to the bell apex and the interradius which is the triangular region between adjacent radial canals.

FIGURE 1 Gross anatomy of *Aequorea victoria*.

Diagram of a hemisphere of *Aequorea* with radial cuts through the margin and bell, showing two perradii. End - endodermal lamella; ExEc - exumbrellar ectoderm; Gon - gonad; INR - inner nerve ring; Man - manubrium; Oc - ocellus; ONR - outer nerve ring; Ped - pédoncle; RdC - radial canal; RdM - radial muscle; Rg C - ring canal; SpM - sphincter muscle; SwM - swimming muscle; Ten - tentacle; Vel - velum.


1cm
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Vel
Ten

The subumbrellar surface is composed of a sheet of circular, striated myoepithelium (swimming muscle). At the perradius, the ectoderm is bilayered with an outer longitudinal, smooth myoepithelium (radial muscle) in addition to the swimming muscle (Plate 1a). A layer of mesoglea, which is thin relative to bell mesoglea, separates the subumbrellar ectoderm from endoderm. Nerves in the subumbrella are limited to the radial nerve bundles on either side of the radial muscle (Plate 1b) and the peripheral radial canal nerves (Plate 1c). In addition, there are scattered, circular, smooth myofibrils at the base of the endodermal cells of the radial canals (Plate 1d).

The primary nervous "center" of hydromedusae consists of the inner (subumbrellar) and outer (exumbrellar) marginal nerve-rings. These nerve bundles are located adjacent to the ring canal at the "tetiradius" where the mesoglea of the subumbrella, exumbrella and velum converge (Plate 2a).

At the margin, the exumbrellar ectoderm is greatly thickened and contains a well-developed array of circular, smooth myofibrils (sphincter muscle) at the mesogleal interface (Plate 2b). A sparse distribution of radial (longitudinal) smooth myofibrils occur at the base of the ectodermal cells of the exumbrellar side of the velum (Plate 2c).

The swimming myoepithelium of the subumbrella continues at the margin almost to the inner nerve-ring and reappears at the base of the velum where it extends to the tip of the velum (Plate 2a, c).

The ring canal contains peripheral nerves and basal circular smooth myofibrils, as in the radial canals.

Although not sectioned, it can be assumed that the manubrium and

tentacles of *Polyorchis* have ectodermal nerve plexuses and longitudinal, smooth myopithelia as reported in other anthomedusae (Kawaguti and Hamakoshi, 1963; Jha and Mackie, 1967; Mackie and Singla, 1975). In addition, there may be an ectodermal nerve plexus in the peduncle as described in *Stomotoca* (Mackie and Singla, 1975).

II. SPECIALIZED MEMBRANE JUNCTIONS IN THE EXCITABLE ENDODERM OF *POLYORCHIS*

a) Verification of Epithelial Excitability

Extracellular recordings from the radial canal and endodermal lamella (Fig. 2, 3) demonstrate conduction of endodermal impulses upon electrical stimulation of the endoderm. Although extracellular action potentials vary greatly in exact form from one recording to recording, the impulses recorded from these two regions of the endoderm have consistently different shapes (Fig. 2, 3). The endodermal lamellar pulse (ELP) consists of an initial quick, negative deflection followed by a slower, larger amplitude negative-going potential and the endodermal canal pulse (ECP) is of a more complex form with an initial shoulder preceding the negative peak, a long duration final negative phase (often flattened) and a pronounced positive after-potential (see Figure 11 for the general pulse forms and Table I for pulse parameters). An important characteristic of endodermal conduction is the passage of excitation between radii (Fig. 2) provided by the presence of electrical connections between lamella and canal. This conduction between radii is associated with a substantial time lag in the appearance of ELPs (Fig. 2) and the conduction velocity observed with

FIGURE 2 Conduction of endodermal lamellar pulses (ELPs).

a) ELPs excited by direct endodermal lamellar stimulation showing conduction within a single radius.

b) Conduction of ELPs between radii showing the delay in condition time associated with excitation transfer between endodermal lamella and radial canal.

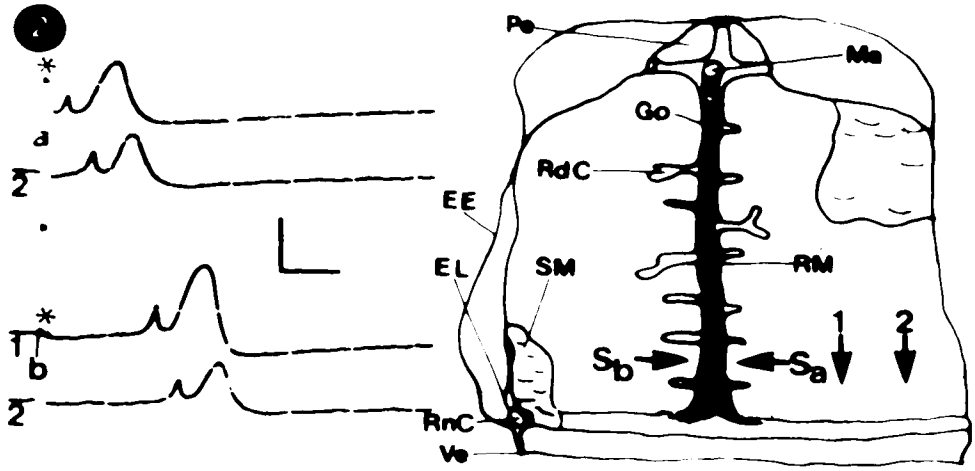
Trace 1 is recording from electrode 1 and trace 2 is recording from electrode 2. Asterisks indicate stimulus artifacts. Diagram symbols: EE - exumbrellar ectoderm, EL - endodermal lamella; Go - gonad; Ma - manubrium, Pe - pedumcle; RM radial muscle; RdC - radial canal; RnC - ring canal, SM - swimming muscle; Ve - velum; S - stimulating electrode; 1 - recording electrode 1; 2 - recording electrode 2; Scale bars - horizontal: 20 ms; vertical: 0.5 mV.

FIGURE 3 Conduction of endodermal canal pulses (ECPs).

a) Excitation of ECPs by stimulation of endodermal lamella recorded from radial canal from which radial muscle has been removed.

b) Distinction between an ECP, recorded as in a) and an ELP resulting from stimulation of endodermal lamella.

Diagram symbols as in figure 2. Scale bars - horizontal: 50 ms; vertical: 2 mV.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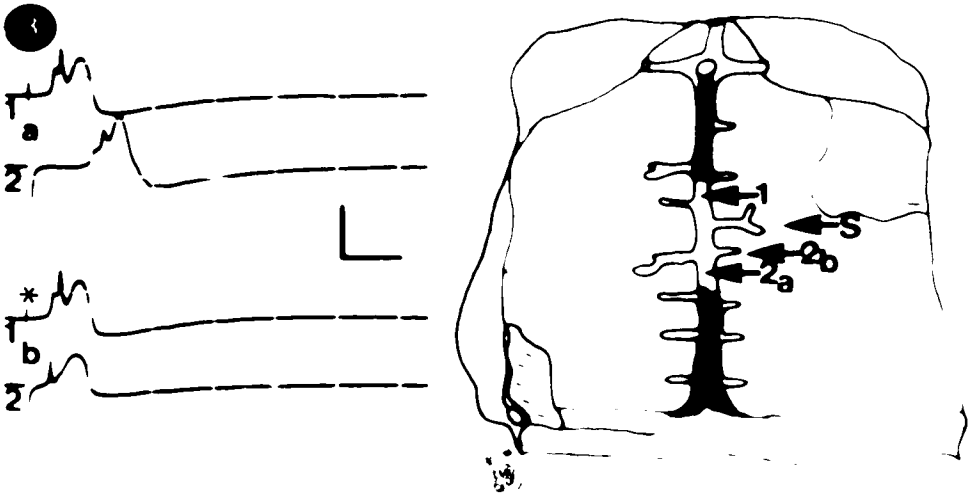
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2a

recording electrodes in different radii is significantly lower than the ELP velocity within a single radius (Table I).

To verify that the excitation recorded from a radial canal is a property of the epithelial cells and not due to the peripheral canal nerves, intracellular recordings have been obtained from the canal (Fig. 4a). These intracellular recordings are comparable to those reported by Spencer (1978). Although the consistency of resting and action potentials and the small size (1-3μm) of the canal nerves makes it virtually certain that these recordings are only from endodermal cells, the possibility exists that nervous activity might in some way contribute to or modify the epithelial potential in the propagation of excitation along the canal. Since no nerves (nor myofibrils) have been located in the endodermal lamella (of any jellyfish), intracellular recordings from this region (Fig. 4b) unequivocally attest to the excitability of endoderm. The mean resting potential of lamellar cells is -67.4mV (S.D. 2.9; n = 52).

In order to investigate the mechanism by which endodermal cells conduct non-decrementing action potentials, intracellular recordings were made from two endodermal lamellar cells simultaneously. Long (600ms) current pulses (of either polarity) through cell 1 cause a deflection of the resting potential of cell 2 (Fig. 5). This demonstrates that the endodermal cells are electrically coupled. However, it was not possible to quantify the efficiency of this coupling by calculating the coupling coefficient (voltage of injected cell 1/voltage of cell 2) due to the inability to record voltage and inject current simultaneously with the same electrode. Although

TABLE I. Parameters of Impulse Types.

	Amplitude (mV)		Duration (ms)				Conduction Velocity (cm/s)	
			Rising		Overall			
	$\bar{x} \pm$ S.D.	n	$\bar{x} \pm$ S.D.	n	$\bar{x} \pm$ S.D.	n	$\bar{x} \pm$ S.D.	n
Endodermal lamellar pulse (ELP)	1.2 ± 0.7	40	22.1 ± 3.2	40	44.2 ± 8.1	40	16.4 ± 4.9[b]	23
							6.4 ± 2.1[c]	9
Endodermal canal pulse (ECP)[a]	1.2 ± 0.5	62	20.4 ± 6.3	60	56.7 ± 9.0	60	16.8 ± 4.7	73
Radial muscle potential (RMP)[a]	2.1 ± 1.1	103	5.5 ± 1.4	74	35.1 ± 7.7	74	21.6 ± 3.6[d]	23
							19.5 ± 4.7[e]	25
							20.5 ± 4.3[f]	48
Exumbrellar pulse (EP)	0.9 ± 0.5	35	7.4 ± 1.3	21	22.3 ± 3.5	20	7.0 ± 1.4	3

[a]Amplitudes and durations for ECPs and RMPs are only from recordings in which the two pulses were clearly distinguished.

[b]Within a single radius.

[c]Between radii.

[d]Marginal pathway.

[e]Apical pathway.

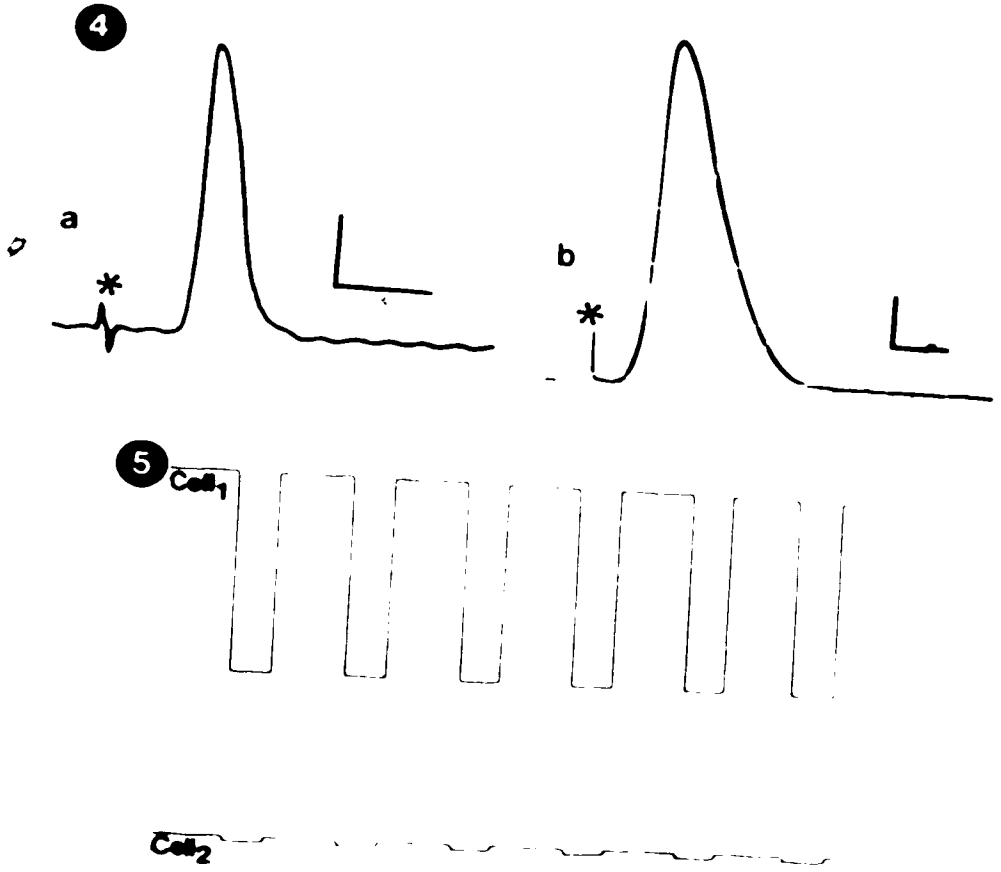
[f]Pooled marginal and apical pathways.

FIGURE 4 Intracellular recordings of endodermal impulses.

a) Intracellular action potential recorded from the radial canal arising from a resting potential of - 59 mV. Extracellular endodermal stimulation. Chart recording. Scale bars - horizontal: 40 ms, vertical: 25 mV.

b) Intracellular action potential recorded from the endodermal lamella arising from a resting potential of -68 mV. Extracellular endodermal stimulation. Scale bars - horizontal: 20 ms; vertical: 20 mV.

FIGURE 5 Electrical coupling of endodermal lamellar cells. Passing 600 ms pulses of hyperpolarizing current (1×10^{-8} A) through cell 1 deflects the resting potential of cell 2 (0.1 mm distant) demonstrating the presence of low-resistance intercellular connections between endodermal lamellar cells. Chart recording.


4
a
b
5
Cell1
Cell2

alternatively it is possible to calculate the space constant of the epithelium by determining the voltage decrement with increasing distance with a constant level of current injection, insufficient two electrode recordings were obtained to permit this estimation.

b) Lanthanum Impregnation of Endoderm

In order to correlate the observed electrical continuity between endodermal cells with specialized structural contacts, endoderm was fixed for electronmicroscopy in the presence of lanthanum (which pervades the extracellular space without penetrating membranes) to outline physical contacts between apposed cells.

(i) Septate junctions

Arrays of septate junctions are prevalent at the lumenal border of the endodermal canals (Plate 3a) and are especially apparent in lanthanum-impregnated material. Although low-magnification micrographs (Plate 3a) suggest a heavy impregnation by lanthanum at septate junctions, higher magnifications (Plate 3b, c) show that there is either an incomplete penetration or that lanthanum was washed out of these regions during subsequent tissue processing. This is contrary to the results of other workers where thorough saturation of septate junctions by lanthanum has been obtained (Hudspeth and Revel, 1971; Hand and Gobel, 1972; Baskin, 1976; Filshie and Flower, 1977). This absence of lanthanum from septate junctions and the general extracellular space appears to be related to the large dimensions of these regions as lanthanum thoroughly and permanently penetrates gap-junctions (Plates 4a, 5, 6) which possess much narrower intercellular spaces.

In transverse section, these septate junctions are characterized by a constant overall width, which varies between junctions from 25-26nm, with an intercellular space of 13-14nm. The septa are regularly spaced with a periodicity of about 14.5nm and are about 7.5nm in width (Plate 3b). Although septa are consistently present in septate junctions, the extent to which they occur along the length of the specialized junctional membrane is variable. The most constant feature of these junctions is the regular spacing of the apposed junctional membrane and the formation of numerous belts enclosing narrow cellular processes or interdigitations (Plate 3a, b, c).

In tangential view, the septate junctions of *Hydra* appear as a series of faint (due to poor lanthanum penetration) concentric curves (Plate 3c). This appearance demonstrates that these septate junctions are of the "*Hydra*-type" as opposed to the honeycomb or pleated arrangement of tangentially sectioned septa in non-coelenterate septate junctions (Danilova *et al.*, 1969; Staehelin, 1974).

Septate junctions are also common in the endodermal lamella, however, no obvious localization within this tissue is detectable.

Within the ectoderm, septate junctions enclosing interdigitations occur at and appear to be restricted to the external surface of the exumbrella (Plate 3d) and subumbrella.

(ii) Gap-junctions

In the endodermal canal, gap-junctions are normally not closely associated with septate junctions. They tend to be concentrated at or near the periphery of the canals (Plate 4a).

In conventionally stained sections these junctions have an intercellular gap of 4-5nm between apposed outer membrane leaflets and an overall width of 14-16nm (Plate 4b).

In tissue impregnated with lanthanum, gap-junctions are more easily recognized and the ultrastructural features are accentuated. It is apparent that lanthanum persists only at gap-junctions, having been washed out of the non-junctional intercellular spaces (Plate 5). The central electron-dense band in these lanthanum-filled junctions is about 7.5-8.5nm. To verify that this distance corresponds to the intercellular space + the two outer membrane leaflets, this region was measured in conventionally stained gap-junctions and is 7-8nm. The overall width of lanthanum filled gap-junctions (15.5-17.0nm) is also comparable to conventionally stained gap-junctions. In certain regions of these transversely sectioned junctions, electron lucent bridges connecting the membranes of adjacent cells can be seen crossing the lanthanum layer (Plate 5b, inset). These lucent bridges have a width of about 6-7.5nm and display a periodicity of about 10-12nm. At some bridges a central electron-opaque line (ca. 2nm wide) can be seen passing between membranes (Plate 5b, inset).

In *en face* sections (parallel to the cell surface) gap-junctions appear as discoidal plaques containing polygonally packed electron-lucent globules with a diameter of about 7.5-8.5nm (Plate 6a, b, c). The spacing of these globules is not as regularly hexagonal as usually described in mammalian tissue (reviewed by McNutt and Weinstein, 1973). Whether this is a characteristic of medusan gap-junctions or is related to their functional state is uncertain. This variance in

spacing is observed between plaques and within a single plaque. Where globules are widely separated lanthanum fills the intervening space and polygonal packing is less obvious (cf. Plate 6c, Plate 6a, b). The center to center distance between globules, when measuring nearest neighbors, is 10-11.5nm. An electron-opaque dot, 2nm wide, is apparent at the center of most globules. At high magnifications many of the globules appear star-shaped (Plate 6c) which suggests they are composed of multiple subunits although photographic rotation would be required to resolve the exact number (Peracchia, 1973).

Gap-junctions are also common in the endodermal lamella but, as is the case for septate junctions, they show no specific localization within this tissue. The tighter association of septate and gap-junctions within the lamella (Plate 6d) is likely a consequence of the smaller size (some 4μm in height as compared to 40μm for endodermal canal cells) of lamellar cells.

Within the ectoderm gap-junctions are especially prevalent between swarming myocytes (Spencer, 1979). They have also been observed within peduncular ectoderm and within exumbrellar and subumbrellar ectoderm at the margin.

c) Freeze-fracture replication of gap-junctional tissue

In addition to the lanthanum impregnation method, freeze-fracture replication is a valuable tool in detailing the fine-structure of specialized membrane junctions (reviewed by Staehelin, 1974; Gilula, 1977). This method allows three-dimensional visualization of the intramembraneous protein particles due to the fact that the lipid layer of membranes in frozen tissue presents the least resistance to cleaving (reviewed by Kobards, 1974).

The use of medusan tissue for freeze fracture imposes complications in the interpretation of fracture faces due to the random nature of the fracture plane and the difficulty of isolating the tissue types prior to freezing and fracturing. Pieces of margin (with the tentacles and velum trimmed down as much as possible) and perradius (with swimming muscle cut as close as possible to the radial muscle) were fractured. It was not usually possible to distinguish ectoderm from endoderm in the replicas.

As splitting a membrane through its lipid bilayer results in two complementary faces, it is important to distinguish between these fracture faces in micrographs. The terminology proposed by Branton *et al.* (1975) is used, whereby the membrane face adhering to the cytoplasm is referred to as the protoplasmic face (PF) and the membrane face closest to the extracellular space the exoplasmic face (EF). The angle of shadowing, which is critical for distinguishing between membrane particles and pits, is indicated in the lower left hand corner of all micrographs.

(i) Septate junctions

Septate junctions are characterized by the presence of rows of EF particles which are convoluted in some regions (Plate 7). At regions where the fracture plane has obviously passed to the apposed cell's plasma membrane, PF grooves complementary to these EF particles are apparent (Plate 7b, c). The septate junctions shown in Plate 7 occur at the surface of the tissue (note the presence of narrow surface projections) and surround cellular interdigitations, substantiating the finding that septate junctions are restricted to sea water-epithelial interfaces.

(11) Gap junctions

Aggregates of intramembraneous particles are common in both the radial and marginal replicas. These can assume a variety of irregular patches or strand. (Plate 8) but usually form distinct plaques (Plate 9).

These membrane particles have diameters of 9.5 11nm and a periodicity of 11 12.5nm. This parallels the measurements of lanthanum impregnated gap junction globules and thus suggests that these particle aggregates in replicas are gap junctions. Note that the slightly larger dimension of the particles of fractured membranes could be attributed to the difficulty of measuring particles that have been shadowed with platinum. Conversely, lanthanum prefers exposed particles which are positively stained around their entire perimeter. In certain replicated particles within the aggregates (Plate 9), a star shaped pit remains and central depressions are variously discernable after the EF as evidenced in lanthanum filled gap-junctions.

These gap-junction particles are located solely on the EF and exceed in diameter the general, non junctional membrane particles which are 7 10nm in diameter. Although gap junction plaques contain regions where the fracture plane deviated from the PF to the EF, no arrays of PF pits complementary to the EF particles have been observed. A correspondence between gap junction particles and pits is of usual occurrence in rat systems.

III. EXCITATION PATHWAYS MEDIATING CRUMPLING BEHAVIOR IN *POLYORCHIS*

a) *Description of the Crumpling Response*

Crumpling behavior in *Polyorchis penicillatus* can be elicited by mechanical or electrical stimulation of any part of the epithelial surface. The exumbrella at or near the margin and any region of the subumbrella are especially sensitive and in an unfatigued animal will invariably evoke crumpling if stimulated.

Full crumpling behavior involves the simultaneous contraction of the four subumbrellar (ectodermal) radial muscles (which may be a stepped response involving numerous twitches) concurrent with contraction of the marginal (ectodermal) sphincter muscle, the longitudinal (ectodermal) muscles of the tentacles and manubrium and the radial (exumbrellar) muscle of the velum. The net result is an involution of the margin, with the retracted tentacles oriented centripetally and upward within the subumbrellar cavity, a shortening and widening of the bell and a narrowing of the bell aperture. Crumpling thus serves to position the delicate tissues (tentacles, nerve-rings, manubrium and gonads) within the partially closed subumbrellar cavity. This presumably acts to protect the animal from a mechanical disturbance, severe enough to excite the epithelial surface, which might represent a potential hazard to the relaxed jellyfish. However, no behavioral studies on any crumpling jellyfish have been made to substantiate the view that this response does indeed protect the animal from intruding organisms. In addition, it has been suggested (Spencer, 1975) that crumpling may serve to transport a negatively buoyant jellyfish from an

attacker by initiating sinking due to the reduced drag of the crumpled animal.

The complexities of the behavior become obvious when a large number of [illegible] have been examined. It appears that although smaller jellyfish will consistently give a strong crumple with mechanical stimulation of any portion of the exumbrella, the medium to large sized animals are less likely to give a full crumple with exumbrellar stimulation. These larger animals often show a partial crumple with exumbrellar stimulation involving retraction of tentacles and radial muscle contraction without a sphincter muscle response. Alternatively, electrical stimulation of exumbrella may stimulate tentacle retraction with the first few stimuli and produce a full crumple with radial and sphincter muscle contractions only after numerous stimuli are given at some critical frequency. These observations immediately suggest that excitation of crumpling may not be entirely epithelial-dependent, occurring in the absence of nervous activity. It is significant that a precedent for nervous intervention during crumpling has been established in two other anthomedusae, [illegible] and [illegible] (Mackie et al., 1967; Mackie and Passano, 1968a). Crumpling in these jellyfish, as in [illegible], can involve a variable response and sphincter contraction is exumbrellar [illegible] during a full crumpling behavior. On the other hand, in the [illegible] medusa, [illegible], which has a more stereotyped crumple response without any evidence of nervous involvement (Mackie, 1964). It may be pertinent that [illegible] lacks a sphincter muscle (Mackie and Mackie, 1975), decreasing the complexity of the response.

[illegible] of the Impulse [illegible] Crumpling

In order to demonstrate the pathways which mediate crumpling, the radial muscle was recorded from with suction electrodes. Activity of this muscle was assumed to be indicative of a crumpling response. Excitation of radial muscle upon subumbrellar stimulation was investigated to determine the means by which endodermal impulses initiate crumpling.

Criteria for distinguishing between the impulse types conducted in the subumbrellar layer must be established before a determination of the epithelial pathways which elicit crumpling can be made. Recordings made with large (25-100 μm) extracellular suction electrodes from an excitable tissue are of variable amplitude, duration and form, dependent upon the electrode tip diameter and amount of tissue sucked into the tip. Activity recorded by suction electrodes attached to radial muscle is especially complex due to the juxtaposition of several excitable tissues: radial muscle, radial nerve, swimming muscle, radial canal and endodermal lamella. To simplify recordings from radial muscle, endodermal lamella was stimulated directly from a region from which the surface layer of swimming muscle was stripped off. This ensured that no excitation of swimming muscle occurred during subumbrellar stimulated crumpling. In addition, the recording electrode tip diameter was kept at or below the width of the radial muscle to limit the probability of recording from endodermal lamella. It can be assumed, therefore, that the subsequent recordings from intact radial muscle during crumpling involve activity

from radial muscle (perhaps summated with radial nerve activity) and radial canal only.

Distinction between radial muscle and endodermal activity was expected to be difficult. On the one hand, these two tissue types are not spontaneously active and it is well established that endodermal excitation occurs concomitant with crumpling (Mackie *et al.*, 1967; Mackie and Passano, 1968; Mackie, 1975; Spencer, 1975, 1978). Radial muscle and endodermal excitation should therefore always occur together. Furthermore, it has been proposed (Mackie *et al.*, 1967; Mackie and Passano, 1968) and substantiated (Mackie, 1975; Mackie and Singla, 1975) that activation of radial muscle occurs through an electrotonic spread from endoderm via epithelial bridges. Hence, it was also anticipated that there would be a tight time relationship between the arrival of endodermal canal pulses (ECPs) and the arrival of radial muscle potentials (RMPs) in recordings from radial muscle.

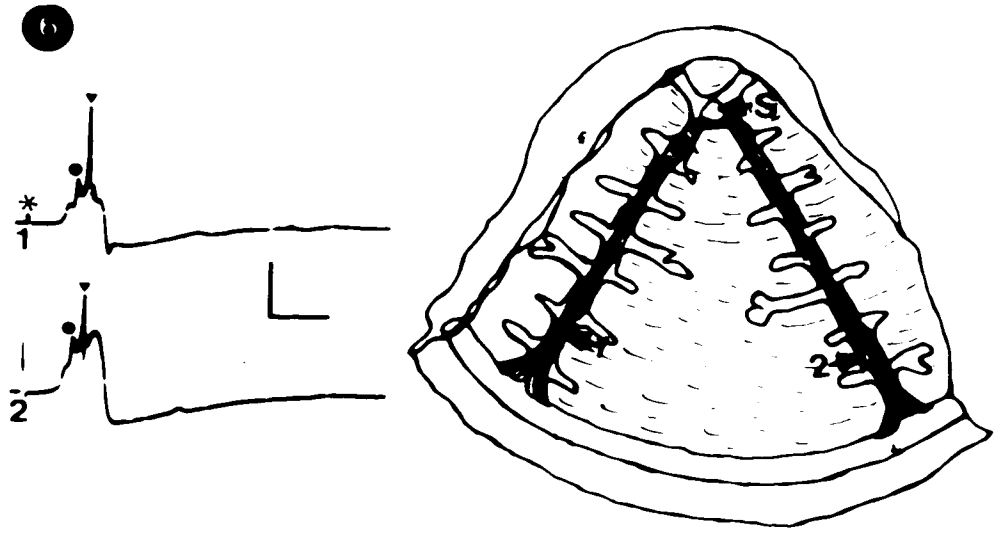
The simultaneous activity recorded from two radial muscles during endodermal stimulation (Fig. 6) show[redacted]e complex (RMP/ECP) potential usually obtained. These records (Fig. 6) involve electrodes positioned on radial muscle equidistant from the stimulating electrode and therefore coincide. It was apparent that recordings from the radial muscle consisted of two separate phases of activity; a slow potential (circle in Figures 6 and 7 and all subsequent figures), which closely resembles ECPs recorded from canal free of radial muscle (Fig. 3a) and a very quick (ca. 5ms rise time) phase usually exceeding the slower potential in amplitude (triangle in Figures 6 and 7 and all subsequent figures). It seemed reasonable that this large, quick event was the radial muscle

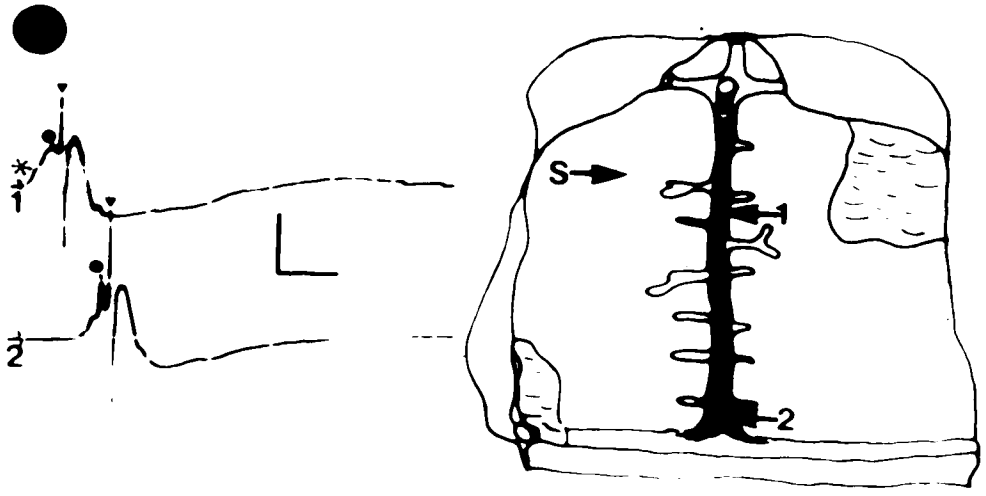
FIGURE 6 Simultaneous crumpling response of two radial muscles.

Complex potentials with slow (circle) and fast (triangle) components recorded from 2 radial muscles during crumpling initiated by stimulating the inner base of the manubrium. Recording electrodes 1 and 2 are equidistant from the margin and the stimulating electrode Symbols as in figure 2. Scale bars - horizontal: 50 ms; vertical: 2 mV.

FIGURE 7 Recording of similar crumpling potentials at two positions on a radial muscle.

Similar complex crumpling potentials recorded at two positions on a radial muscle upon stimulation of endodermal lamella. The response appears first at electrode 1 which is closer to the stimulating electrode. The slow (circle) and fast (triangle) components appear at the same phase of the response in both records. Symbols as in figure 2. Scale bars - horizontal: 50 ms; vertical: 2mV.


6
1
2
S
1
2


1
2
S
1
2

potential. The compound potential recorded from radial muscle often had a latency directly proportional to the distance of recording electrodes 1 and 2 (R1 and R2) from the stimulating electrode (S), with the quick event occurring at the same phase of the potential at R1 and R2 (Fig. 7). Other cases were obtained in which the quick event appeared at different phases of the crumpling potential at R1 and R2 although the latency of the response initiation was again proportional to the distance of R1 and R2 from S (Fig. 8a, b). This was enigmatic because it is expected that there would be a tight relationship between the appearance of ECPs and the appearance of RMPs at all recording sites if endoderm directly excites radial muscle.

Before resolving this ambiguity, it is necessary to confidently distinguish the ECP from the RMP phase of the complex response recorded from radial muscle during crumpling. The approach used was to compare the compound crumpling potential with activity of isolated radial canal and isolated radial muscle. The usual compound event recorded from intact radial muscle (Fig. 9a) becomes simplified when the underlying radial canal was recorded from after the radial muscle and radial nerves were stripped from the region (Fig. 9b). This demonstrates that the slow, long duration component of the complex event is indeed the ECP (see Table I for impulse parameters). The complementary experiment involved isolation of radial muscle from the radial canal. Prior to dissection, a typical RMP/ECP complex is recorded from R1 and R2 (Fig. 10a). Note that the slow and quick phases are dissociated here because the muscle was cut apically for reasons that will become apparent. When a portion of the radial

FIGURE 8 Recording of crumpling potentials which differ at two positions on a radial muscle.

Complex crumpling potentials recorded at two positions on a radial muscle which differ in waveform. In these recordings resulting from endodermal lamellar stimulation the fast (triangle) component occurs at different phases of the slow (circle) potential. In a) the response appears first at recording electrode 2 which is closer to the stimulating electode. In b) the slow potential arrives first at the electrode closer to S (R2) but the fast potential arrives first at R1 which is closer to the margin. The apical attachment of the radial muscle was inadvertently damaged (semicircle). Symbols as in figure 2. Scale bars - horizontal: 50 ms; vertical: 2 mV.

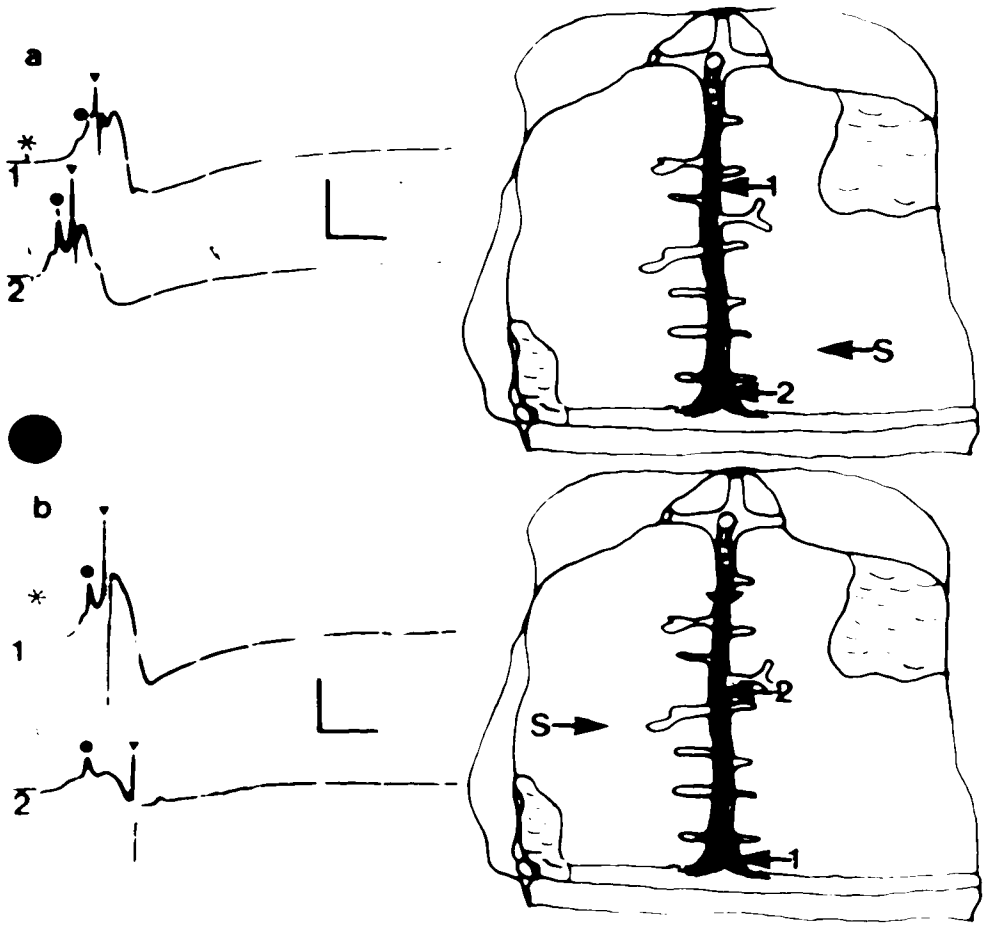

a
*
1
2
S
1
2
b
*
1
2
S
2
1

FIGURE 9 Determination of the endodermal canal pulse (ECP) component of the crumpling potential.

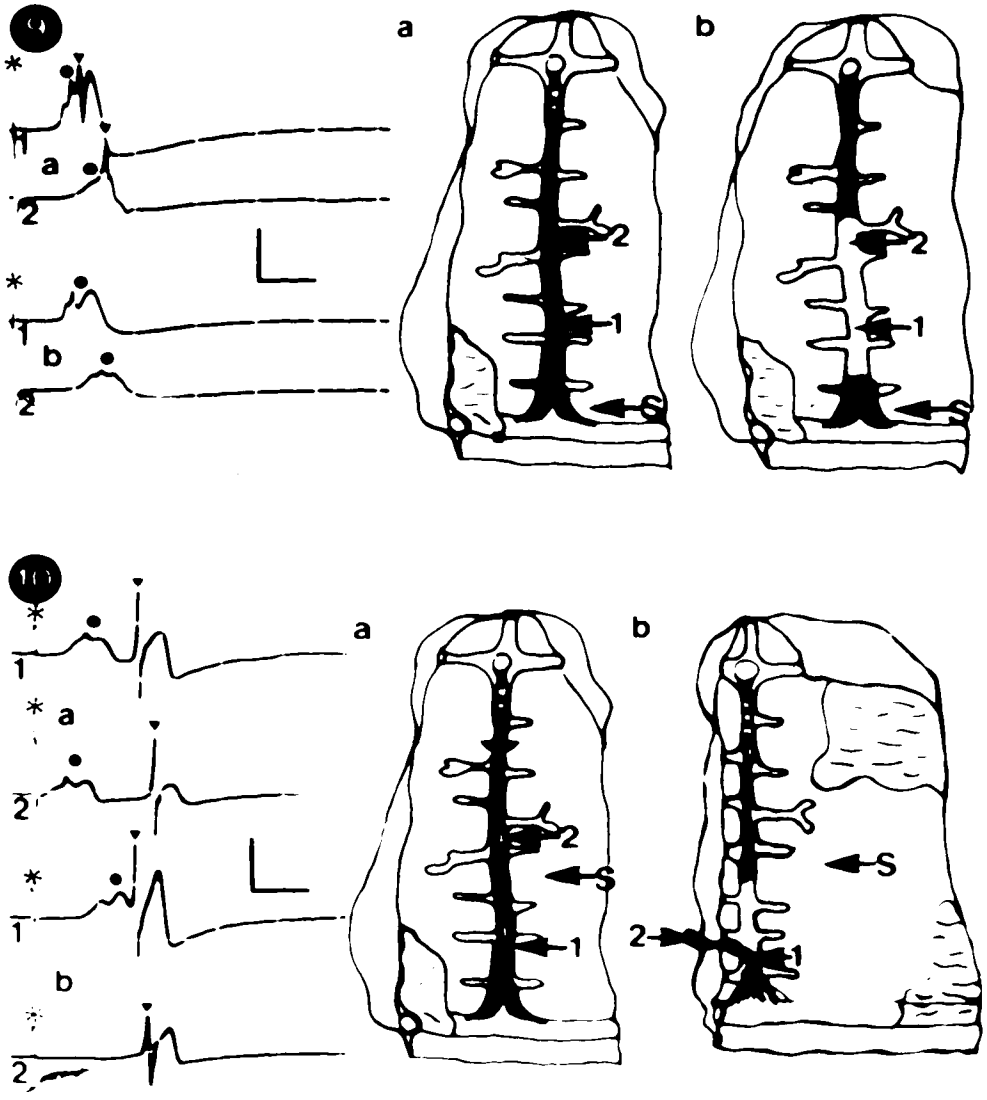
a) Complex crumpling potentials recorded from intact radial muscle with endodermal lamellar stimulation.

b) Recording from the radial canal after radial muscle is peeled off showing that the slow component (circle) of the potential in a) is the endodermal canal pulse (ECP) and suggesting that the quick component (triangle) in a) is the radial muscle potential (RMP). Symbols as in figure 2. Scale bars - horizontal: 50 ms; vertical: 2 mV.

FIGURE 10 Determination of the radial muscle potential (RMP) component of the crumpling potential.

a) Complex crumpling potentials recorded from intact radial muscle with endodermal lamellar stimulation. The apical connection of the radial muscle has been cut (semi-circle).

b) Comparison between a complex crumpling potential, showing the slow (circle) and fast (triangle) components, from intact radial muscle (R1) and pure radial muscle potential (RMP) from isolated radial muscle (R2). The fast component (triangle) of the crumpling response is therefore the RMP. Symbols as in figure 2. Scale bars horizontal: 50 ms; vertical: 2 mV.


9
a
b
1
2
S
10

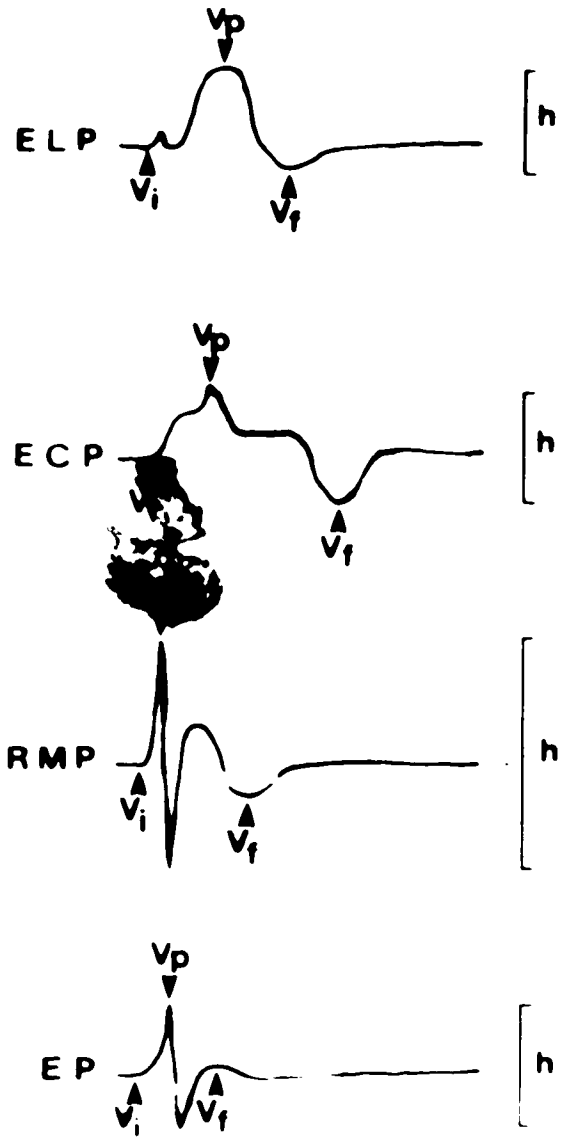
muscle is peeled from the canal, with its attachment to the margin intact, only the quick phase is picked up by R2 attached to isolated muscle although the compound event is recorded from R1 attached to intact muscle (Fig. 10b). This is conclusive evidence that the quick, larger amplitude component of the complex crumpling event is the RMP (see Table I for impulse parameters).

Although these experiments clearly discriminate between the ECP and RMP from isolated tissues, separation of these two potentials in recordings from intact radial muscle is hindered by the inherent variation in extracellular recordings and the inconsistent latencies of the RMPs. In order to determine whether the impulse parameters of ECPs and RMPs (Table I) are significantly different a statistical analysis was performed. The standard test of significance between the means of two randomly selected data populations is the student t-test. This test showed that the difference between peak-to-peak amplitude, rising and overall duration (see Figure 11 for definitions of rising and overall durations) between ECPs and RMPs is highly significant ($p < 0.0005$). However, it could be argued that such a parametric statistical test is inappropriate for analysis of this data due to the large difference in variance between the two data populations. A more satisfactory test is the Mann-Whitney U test (Miller and Freund, 1977) which makes no assumptions as to the normal distribution or differences in variance of populations. Calculation of the U value demonstrated that there is a highly significant difference ($p < 0.005$) between the peak-to-peak amplitude, the duration of the rising phase and the overall duration

FIGURE 11 Impulse waveforms and definitions of their electrical parameters.

Diagrams of characteristic waveforms of the endodermal lamellar pulse (ELP), endodermal canal pulse (ECP), radial muscle potential (RMP) and exumbrellar pulse (EP). Rising duration is defined as the time between potential initiation (V_i) and peak negative potential (V_p) and overall duration is defined as the time between potential initiation and peak after-potential (V_f). Impulse amplitudes were measured as the magnitude of negative peak to positive peak potential (h).

11



of ECPs and RMPs. It is, therefore, valid to distinguish the two phases of the complex event recorded from radial muscle solely on the basis of differences in impulse amplitude and duration. Differentiation between the endodermal and radial muscle elements of the crumpling event then allows an ascertainment of the conduction pathways exciting radial muscle by comparing the relationship between the response latency and point of stimulation for RMPs and ECPs recorded from the radial muscle.

c) Excitation Pathways Active during Subumbrellar [illegible] of Crumpling

Referring back to Figure 8b, RMPs recorded by [illegible] at different phases of the compound crumpling event. [illegible] expected the ECP arrives sooner at R2 than at R1 since the stimulating electrode is closer to R2. However, as noted before, the RMP arrives first at R1. This suggests that the endodermal lamellar pulse (ELP) must pass to the margin in order to initiate an RMP and that this muscle excitation is then conducted towards the apex.

In order to confirm that marginal excitation of radial muscle occurs, the effect of altering the position of S while R1 and R2 remain stationary was tested (Fig. 12). When the endodermal lamella is stimulated at a position near the margin (Fig. 12a) the latency of RMPs is dependent on the distance of R1 and R2 from the margin, i.e. radial muscle activity is initiated at the margin. However, movement of S towards the apex has an unanticipated effect (Fig. 12b). Rather than simply delaying the time of arrival of the crumpling event, this apical positioning of S causes the latency

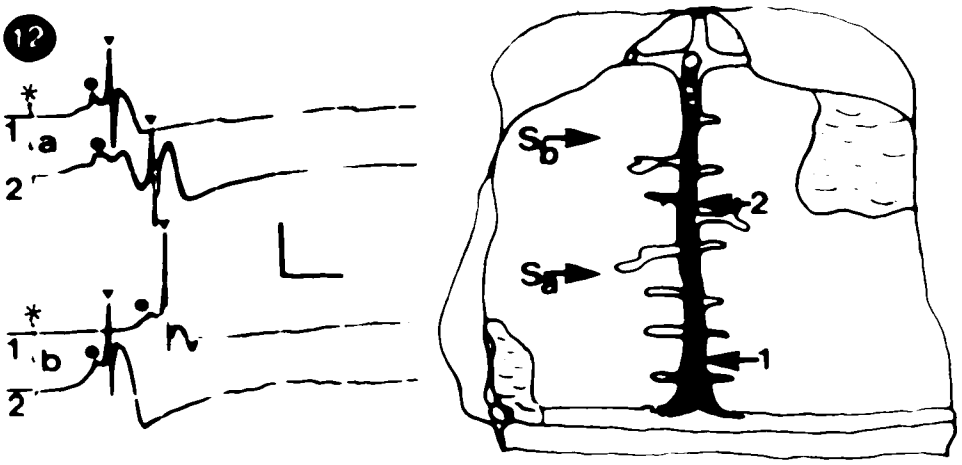
FIGURE 12 Demonstration of marginal and apical EPP initiation sites.

a) Stimulating the endodermal lamella equidistant between R1 and R2 and between margin and peduncle excites an ICP (circle), recorded simultaneously from R1 and R2, and an EPP (triangle), arriving first at R1. Radial muscle activity is initiated at the marginal end of the muscle.

b) Stimulating apical endodermal lamella, with R1 and R2 as in a), excites an ICP and EPP which both arrive first at R2, showing that radial muscle can also be activated at its apical end. Symbols as in figure 2. Scale bars - horizontal: 50 ms; vertical: 2mV.

FIGURE 13 Demonstration of a distinct apical EPP pathway. When a radial muscle is cut between margin and apex, apically directed EPPs are recorded from the marginal portion of muscle upon apical endodermal lamellar stimulation a) and marginally directed EPPs are recorded from the apical portion of muscle upon marginal endodermal lamellar stimulation b). The EPP (triangle) arrives first at R2 despite the earlier appearance of the ICP (circle) at R1, showing apical excitation is not via endoderm. Symbols as in figure 2. [illegible] marks radial muscle fibre. Scale bars - horizontal: 50 ms; vertical: 2 mV.

12



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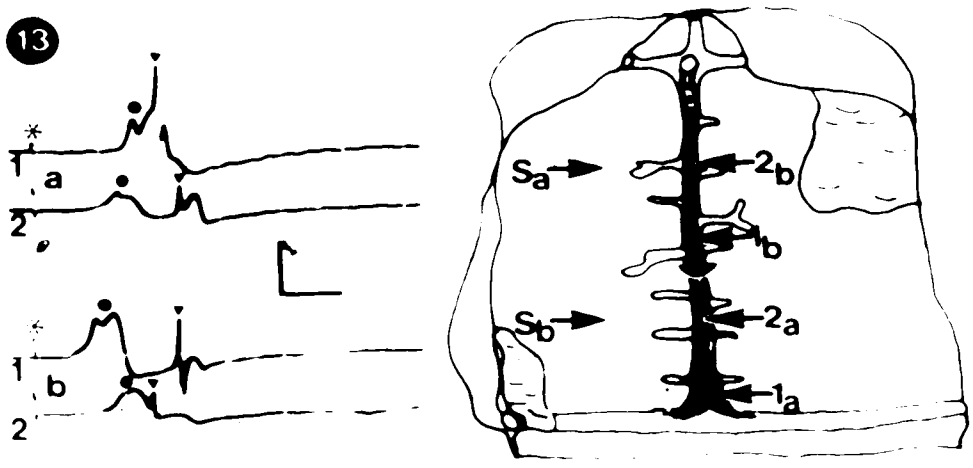


of the RMP at R2 to markedly decrease such that it precedes the arrival of the RMP at R1, the electrode nearest the margin. This result can be accounted for in two ways; either apical stimulation produces a direct endodermal excitation of radial muscle (note that the ECP of R2 in Figure 12b arrives before the ECP of R1) or there exists an apical RMP pathway in addition to the marginal pathway.

To discriminate between these two possible mechanisms of apical radial muscle activation, radial muscle was cut midway between its marginal and apical attachments (Fig. 13). Since the two segments of muscle each have an intact connection at opposite ends, this allows a separation of the marginal and apical excitation routes within a single radial muscle. Endodermal stimulation produces RMP conduction towards the apex in the marginal portion of the muscle (Fig. 13a) and RMP conduction towards the margin in the apical portion of the muscle (Fig. 13b). Because S is distant from the site of RMP initiation in both cases, a wide dissociation occurs between the ECP and RMP at the more distal recording electrode (R2 in Figure 13a and R1 in Figure 13b). This disjunction of the ECP and RMP in R1 of Figure 13b indicates that the apical initiation of crumpling does not involve a direct endodermal excitation of radial muscle but that there must be a distinct apical pathway in addition to the marginal RMP pathway.

Experiments were then made to substantiate the independence of endodermal and radial muscle conduction. In order to simplify the analysis, preparations were often used in which one of the conduction pathways was destroyed. In demarginate preparations, the radial muscle and radial nerves were severed at the margin and in

depedunculate preparations, the radial muscle and nerves were severed apically at the base of the peduncle. Both of these operations were done without damaging the continuity of the underlying radial canal.

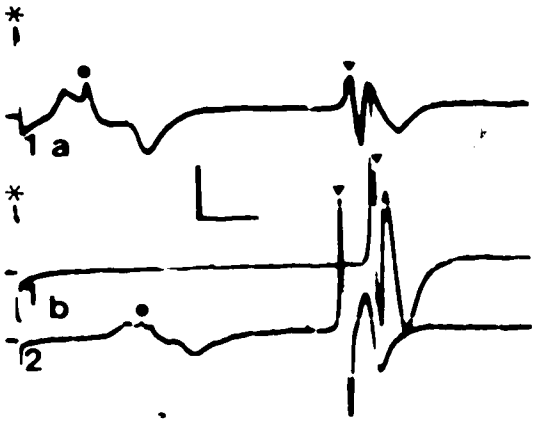
Radial muscle was isolated from the endoderm by cutting the endodermal lamella and radial canal around a region of muscle in a demarginate preparation in order to determine whether an RMP would conduct along a muscle in the absence of endoderm (Fig. 14). Endodermal stimulation elicits crumpling which is associated with an ECP and RMP recorded from intact radial muscle prior to dissection (Fig. 14a) and from an intact region of muscle after dissection (R2 in Figure 14b). Removing the radial muscle from contact with endoderm blocks the appearance of ECPs but does not inhibit RMP propagation (Fig. 14b).

Additional cutting experiments were performed to verify that initiation of the radial muscle response requires connection of the muscle to the margin (for the marginal pathway) and to the peduncle (for the apical pathway). The marginal conduction of RMPs in a depedunculate preparation (Fig. 15a) is blocked when the marginal attachment of the muscle and the radial nerves are also severed (Fig. 15b). Similarly, the apical conduction of RMPs in a demarginate preparation (Fig. 16a) is prevented by cutting the connection of the radial muscle and nerves at the base of the peduncle (Fig. 16b). In both of these experiments the conduction of endodermal impulses is unaltered and the observation of sphincter muscle contraction associated with this endodermal excitation shows that crumpling is still activated.

FIGURE 14 RMP conduction in the absence of endoderm.

a) Recording of an ECP (circle) and RMP (triangle) from an intact region of radial muscle in a demarginate preparation. This recording was made before dissection at about the position of R1 in b).

b) RMPs conduct through regions of radial muscle isolated from the underlying endoderm. No ECP is recorded from R1 although an ECP and RMP is recorded by R2 on intact radial muscle a short distance away. Symbols as in figure 2. Semi-circle marks radial muscle incision muscle. Scale bars - horizontal: 50 ms; vertical: 1 mV.



14

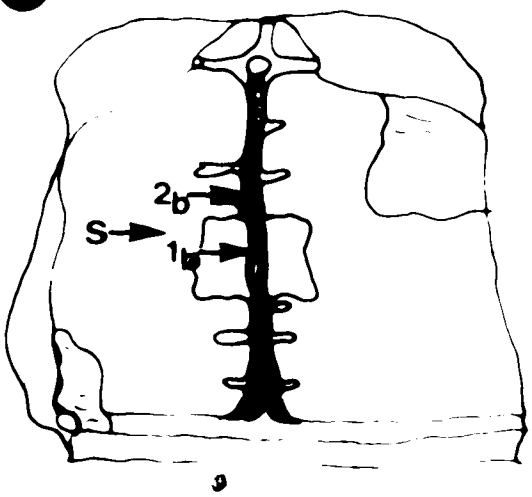


FIGURE 15 Requirement of marginal RMP pathway for radial muscle attachment to the margin.

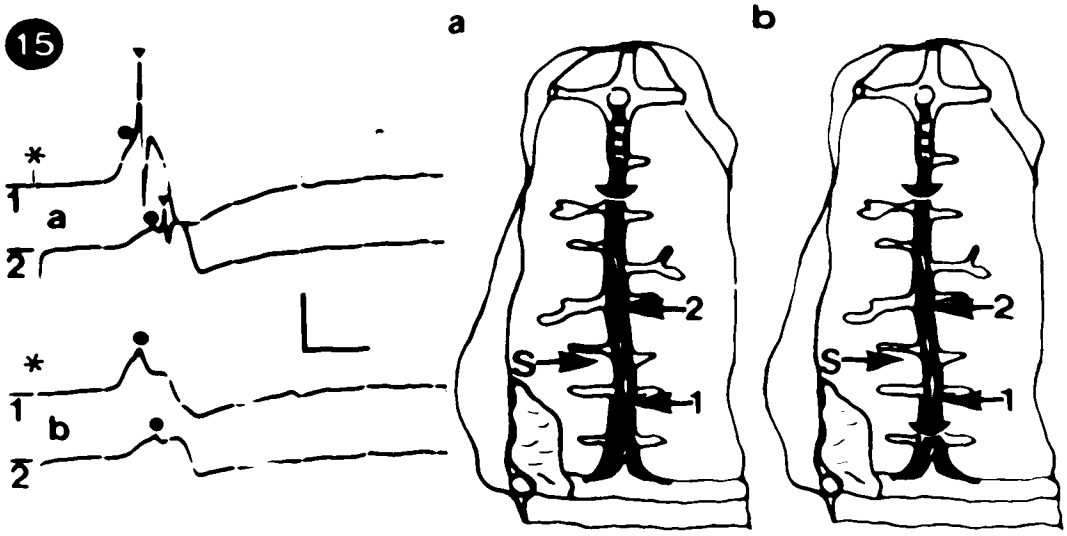
a) RMPs (triangles) conducted from the margin and ECPs (circles) recorded from radial muscle in a depedunculate preparation with endodermal lamellar stimulation.

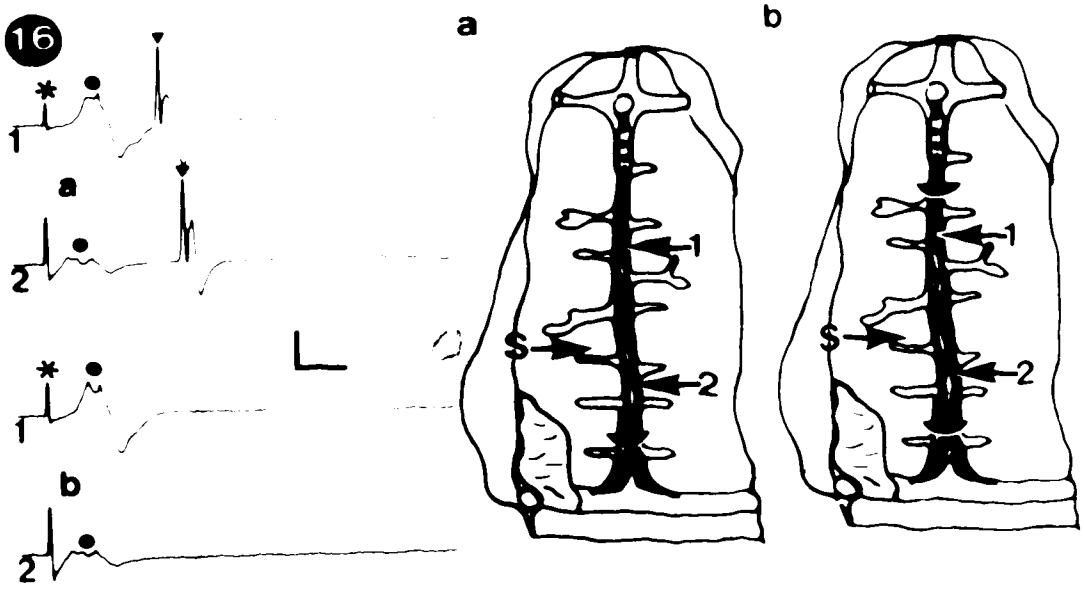
b) ECPs recorded from radial muscle after marginal attachment of the muscle is cut (semi-circle). Initiation of RMPs is blocked. Symbols as in figure 2. Semi-circles mark radial muscle incisions. Scale bars - horizontal: 50 ms; vertical: 2 mV.

FIGURE 16 Requirement of apical RMP pathway for radial muscle attachment to the base of the pedunicle.

a) RMPs (triangles) conducted from the apex and ECPs (circles) recorded from radial muscle in a demarginate preparation with endodermal lamellar stimulation.

b) ECPs recorded from radial muscle after apical attachment of the muscle is cut (semi-circle). Initiation of RMPs is blocked. Chart recordings. Symbols as in figure 2. Semi-circles mark radial muscle incisions. Scale bars - horizontal: 50 ms; vertical: 2 mV.


15
a
b
*
1
a
2
*
1
b
2
S
1
2
S
1
2


16
a
b
*
1
a
2
*
1
b
2
S
1
2
S
1
2

To further demarcate the two RMP pathways the conduction parameters of restricted (demarginate or depedunculate) and intact preparations were investigated. Conduction velocities of RMPs were calculated by dividing the distance separating two recording electrodes by the time difference of their RMPs. Estimates of conduction velocities for the two RMP pathways can be obtained only in restricted preparations where the excitation pathway is certain. It is apparent that the difference between the mean conduction velocities for the two pathways is negligible (Table I). There is, however, a distinction between RMP latencies when comparing the crumpling events of a restricted (Fig. 17) and an intact (Fig. 18) preparation. If only one pathway exists to excite the radial muscle, the difference in conduction time between R1 and R2 remains constant when S is moved (Fig. 17a-d). Alternatively, if both pathways are available to activate RMPs, the difference in conduction time between R1 and R2 is variable and a certain critical positioning of S will cause the RMPs of R1 and R2 to superimpose (Fig. 18a-d). For a statistical treatment, the means of the RMP conduction velocities for marginally and apically directed conduction of each preparation type (restricted and intact) were pooled. Note that for the intact preparations this measurement results in an "apparent" conduction velocity because differences in the RMP latencies between R1 and R2 are due to the presence of two pathways and not to a change in the velocity of RMP conduction. Use of the Whitney-Mann U test (Miller and Freund, 1977) demonstrated that there is a significant difference ($p > 0.05$) between the mean RMP conduction times of restricted and intact preparations (Table II). In addition, there is a highly

FIGURE 17 RMP conduction times in a restricted preparation. RMPs (triangles) and ECPs (circles) recorded from a depeduncular radial muscle at four (a-d) stimulating positions on the endodermal lamella. As S is moved apically the response latency of the RMP increases. The difference in conduction time between RMPs at R1 and R2 remains constant at all stimulation sites. Symbols as in figure 2. Semi-circle marks radial muscle incision. Scale bars - horizontal: 50 m.; vertical: 2 mV.

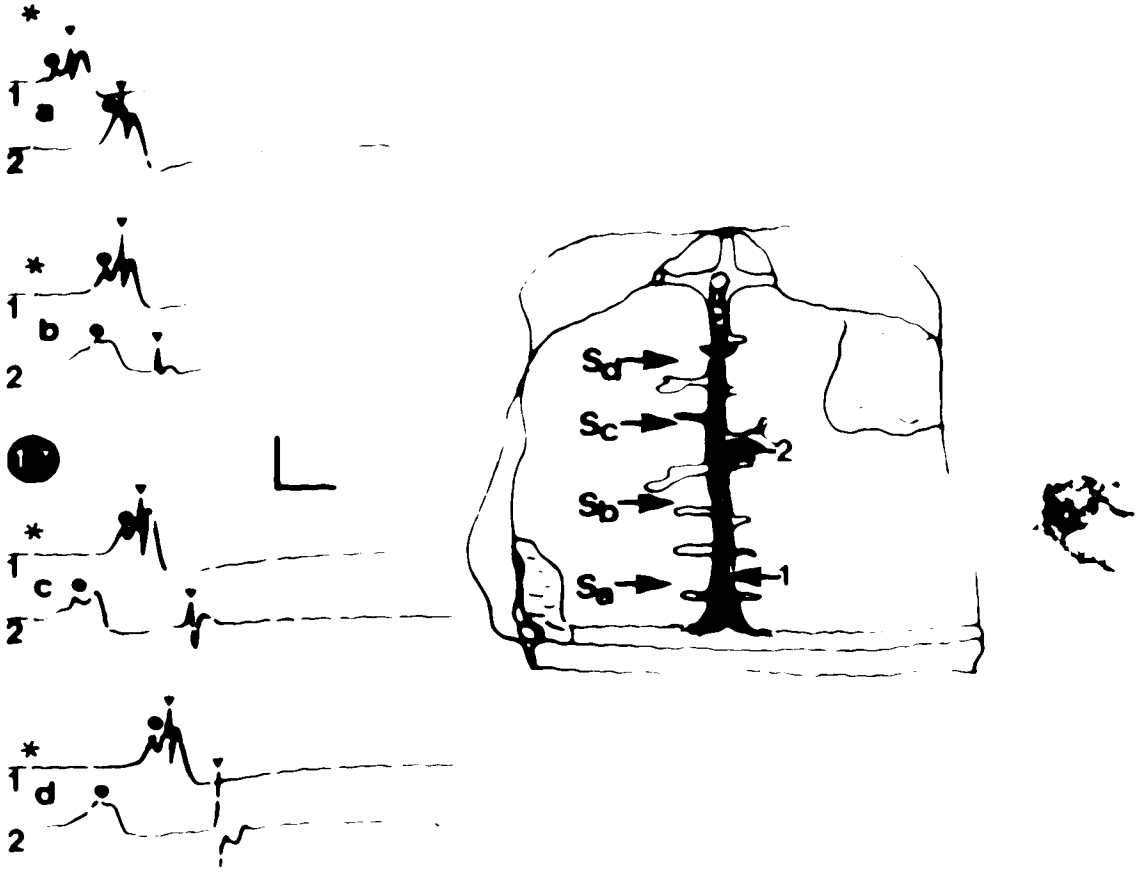

a
b
c
d
1
2
S_d
S_c
S_b
S_a

FIGURE 18 RMP conduction times in an intact preparation.

RMPs (triangles) and ECPs (circles) recorded from an intact radial muscle at four (a-d) stimulating positions on the endodermal lamella. In a) and b) RMPs arrive first at the electrode nearest the margin (R1) since S is close to the margin. In c) and d) RMPs appear first at the more apical electrode (R2) since S is close to the peduncle. The difference in conduction time between RMPs at R1 and R2 is variable due to the possible initiation of RMPs at both ends of the radial muscle especially apparent in b). Symbols as in figure 2. Scale bars - horizontal: 50 ms; vertical: 2 mV.

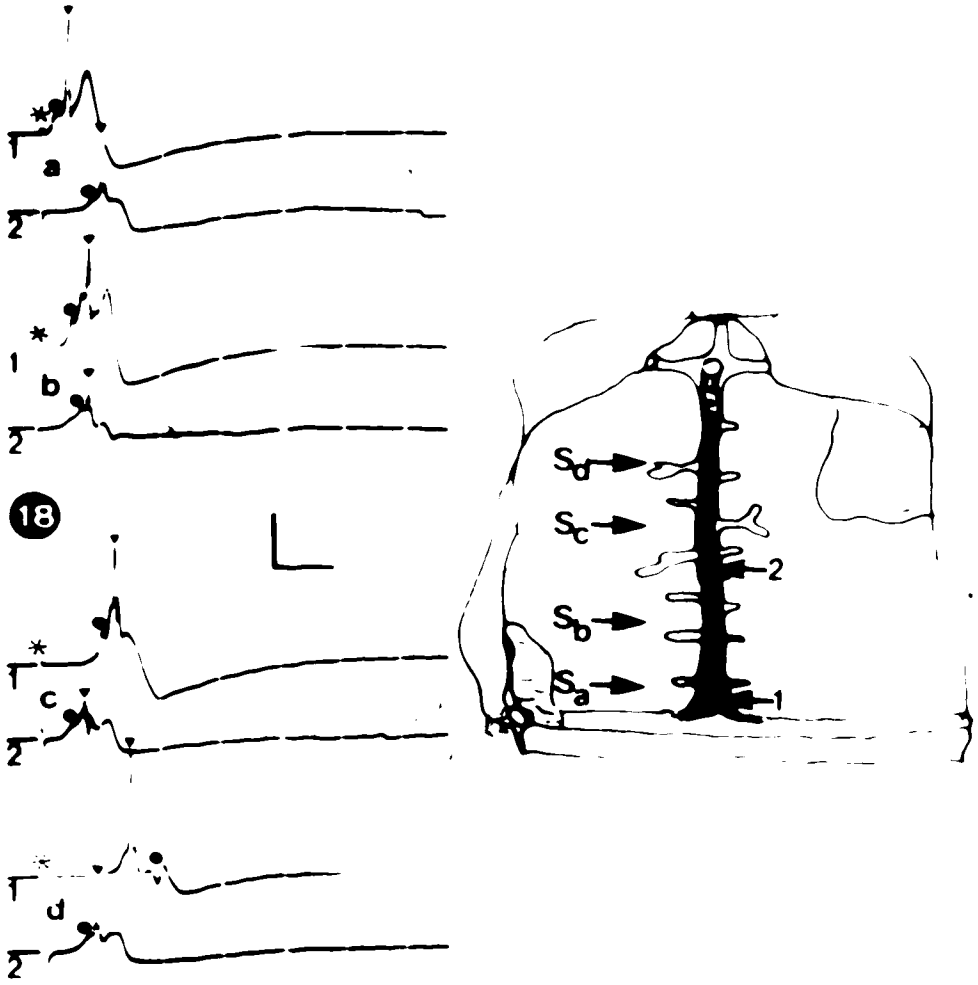

i
a
2
1
b
2
18
L
i
c
2
d
2
S_d
S_c
2
S_b
S_a
1

TABLE II. Conduction Times of Radial Muscle Potentials.

Restricted Preparation

	Conduction velocity (cm/s) $\bar{x}$ ± S.D.	n
Marginal pathway	21.6 ± 3.6	23
Apical pathway	19.5 ± 4.7	25
Pooled pathways	20.5 ± 4.3	48

Intact Preparation

	"Apparent" conduction velocity (cm/s)* $\bar{x}$ ± S.D.	n
	29.1 ± 18.4	36

* These measurements are not true conduction velocities because they involve conduction of RMPs in two directions (see text).

significant difference ($p < 0.005$) between EMP (pooled depedunculate and demarginate restricted preparations) and ECP conduction velocities (Table I).

Another possible criterion for distinguishing conduction pathways is to determine their rate of fatigue with repetitive stimulation. Repetitive stimulation of apical epidermal lamella excites radial muscle through the apical pathway with the first shock but each successive stimulus produces a marginally activated muscle response (Fig. 19). When one recording electrode is attached to radial muscle near the margin, it is apparent that only a marginal conduction of RMPs occurs with the second stimulus (Fig. 19a). Alternatively, when both recording electrodes are distal from the margin it is apparent that RMPs are initiated at both ends of the radial muscle (Fig. 19b). This ambiguity is resolved by observing RMP fatigue in restricted preparations (Fig. 20). Comparing the rate of RMP fatigue of the marginal pathway in a depedunculate preparation (Fig. 20a) with that of the apical pathway in a demarginate preparation (Fig. 20b) shows that the marginal pathway fatigues more slowly than the apical pathway. Note that the fatigue of the apical route involves an increased initiation delay of RMPs with each stimulus although the conduction velocity remains fairly constant (Fig. 20b). Almost no increase in initiation delay with repetitive stimulation is discernable for the marginal RMP route (Fig. 20a).

Stimulation of the surface of the margirium excites the radial muscle via the apical pathway. This activation of crumpling is presumably mediated through the endoderm as severing the radial

FIGURE 19 Differential fatigue of the two RMP pathways in an intact preparation.

Raster tracings involving consecutive sweeps (a-d) of R1 and R2, each separately triggered by a stimulus.

Apical initiation of RMPs (RMP of R1 precedes that of R2) resulting from ectodermal basilar stimulation near the peduncle - facilitated by repetitive stimulation at 0.2 pulses/s (pps). Second stimulus in a) excites radial muscle through the marginal pathway (RMP of R2 precedes that of R1). In b) the RMPs of R1 and R2 superimpose showing activation of both ends of the muscle. The apical pathway is therefore more labile that the marginal pathway. Symbols as in figure 2. RMP - triangle, EOP - circle. Scale bars - horizontal: 50 ms; vertical: 1 mV.

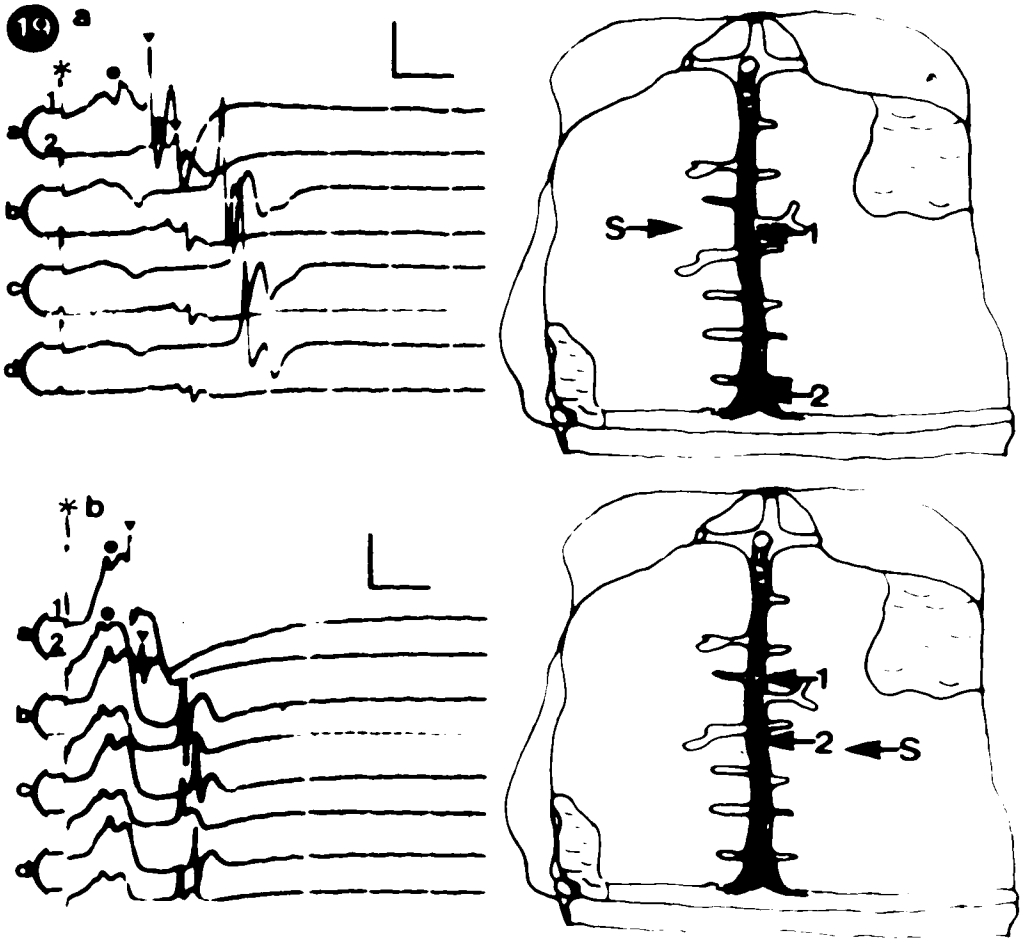
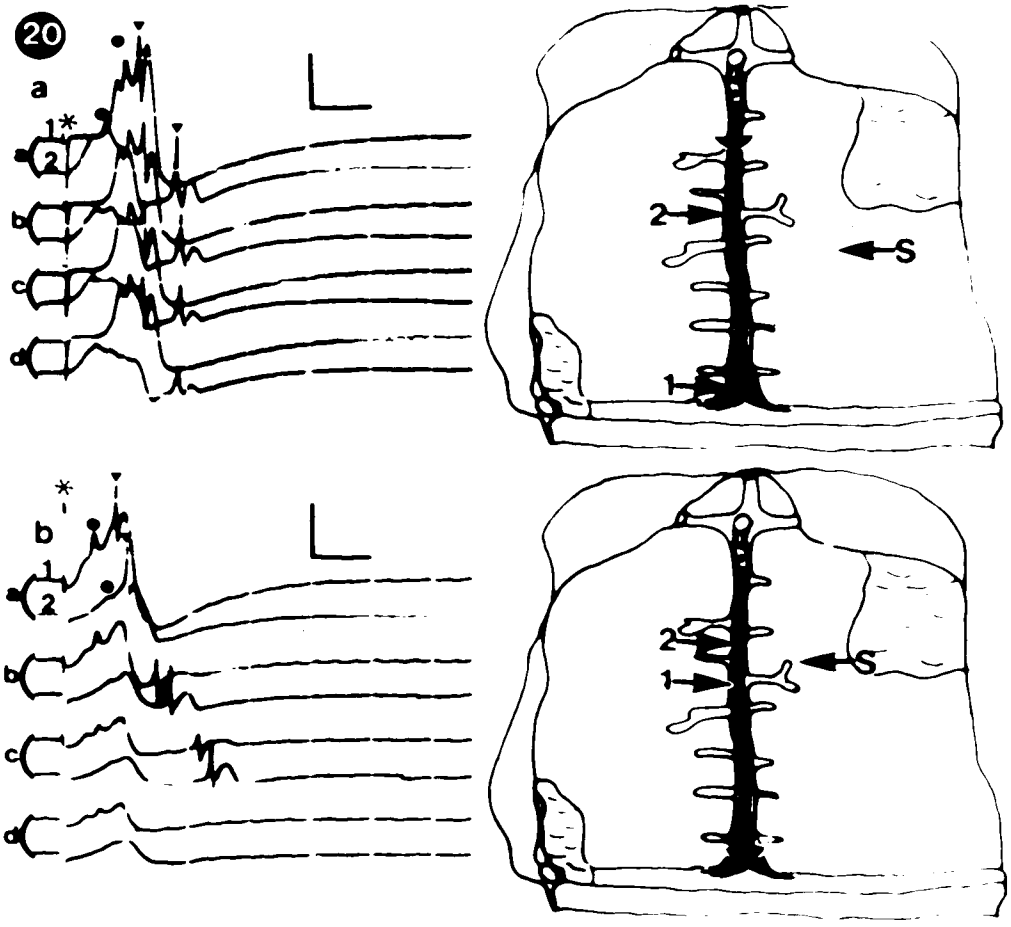

19
a
S
2
b
1
2
1
2
S

FIGURE 20 Fatigue of the marginal and apical RMP pathways in restricted preparations.

Raster tracings involving consecutive sweeps (a-d) of R1 and R2 each separately triggered by a stimulus.

a) Repetitive stimulation at 0.2 pulses/s (pps) of endodermal lamella in a depedunculate preparation results in a minimal fatigue of RMPs (triangles) initiated at the margin. FCPs (circles) show a similar rate of fatigue.

b) Repetitive stimulation at 0.2 pps of endodermal lamella in a demarginate preparation results in a substantial fatigue of apically initiated RMPs. This involves an increased initiation delay of RCPs and the fourth shock fails to activate the muscle. Symbols as in figure 2. Semi-circles mark radial muscle incisions. Scale bar - horizontal: 50 ms, vertical: 1 mV.


20
a
1*
2
b
c
d
2
S
1
b
*
1
2
b
c
d
2
1
S

ectoderm between the manubrium and the base of the peduncle does not block RMPs. However, it was not possible to separate manubrial ectoderm from endoderm to determine whether excitation could pass along the peduncular ectoderm to the apical RMP initiation site.

d) Excitation Pathways Active during Exumbrellar Stimulation of Crumpling:

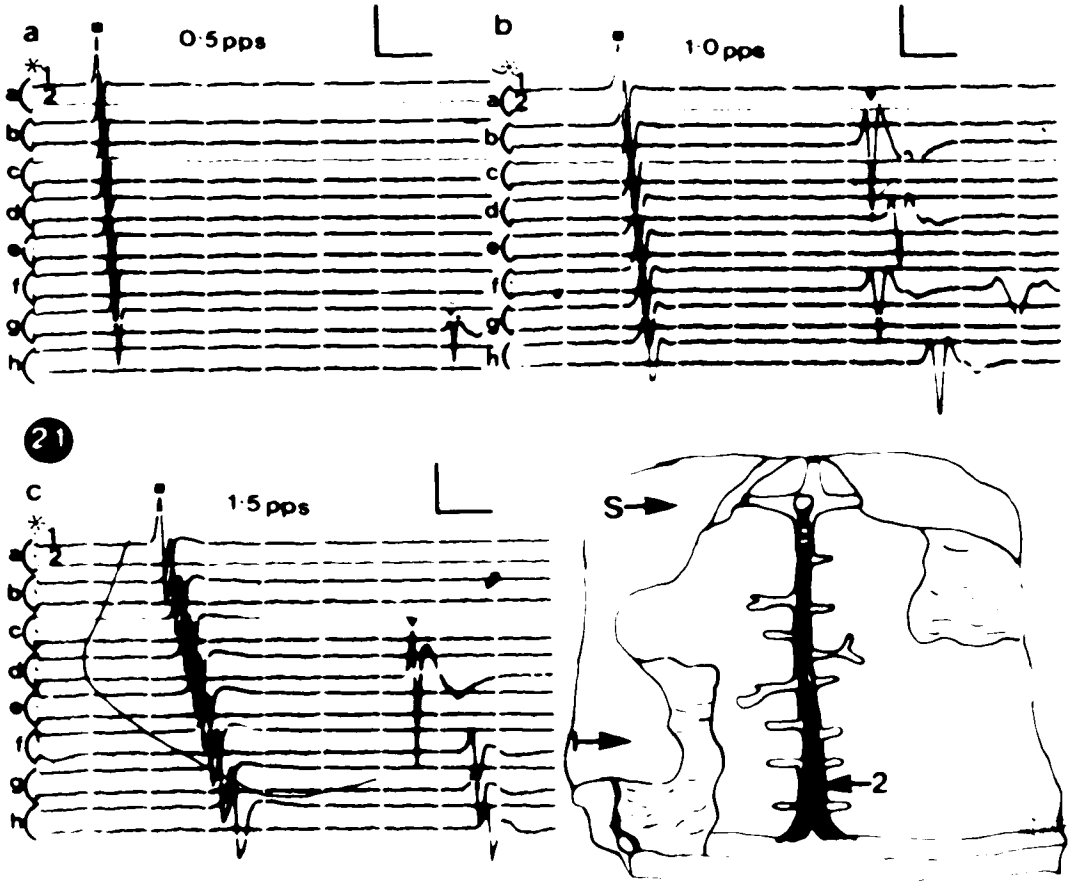
The crumpling response resulting from electrical stimulation of the exumbrellar ectoderm is more variable than the response to subumbrellar stimulation. Exumbrellar excitation usually provokes tentacle retraction, variably activates radial muscle response and only occasionally elicits sphincter muscle contraction. The activity of the latter effector defines a "full crumpling response" which is invariably initiated by subumbrellar stimulation.

Exumbrellar activation of crumpling is associated with the conduction of exumbrellar pulses (EPs) which differ markedly from endodermal pulses (ECPs and ELPs) in terms of impulse form and duration (Table I and Fig. 11) despite the histological similarity of the exumbrellar ectoderm and the endodermal lamella. Eventhough EPs consistently result from exumbrellar stimulation, there is usually not a one-to-one relationship between EP conduction and RMP excitation (Fig. 21). Lower stimulus frequencies generally require more EPs to excite RMPs (Fig. 21a) than higher frequencies (Fig. 21b, c) although no tight correlation between the rate of stimulation and the number of EPs required is apparent (cf. Fig. 21b and 21c). In addition, this correlation is highly variable between animals.

FIGURE 21 Requirement of RMPs for facilitation of exumbrellar pulses (EPs).

Raster tracings involving consecutive sweeps (a-h) of R1 and R2 each triggered by a separate stimulus.

RMPs (triangles) excited by EPs (squares) as a result of repetitive stimulation of the exumbrellar ectoderm at 0.5 pulses/s (pps) in a) 1.0 pps in b) and 1.5 pps in c). Activation of radial muscle requires more EPs at lower frequencies a) but stimulation at 1.5 pps in this case requires more EPs than stimulation at 1.0 pps. Initiation delay of EPs is increased at higher stimulus frequencies c). Symbols as in figure 2. Scale bars - horizontal: a) 100 ms, b) c) 50 ms; vertical: 1 mV.


a
0·5 pps
b
1·0 pps
c
1·5 pps
S
2
21

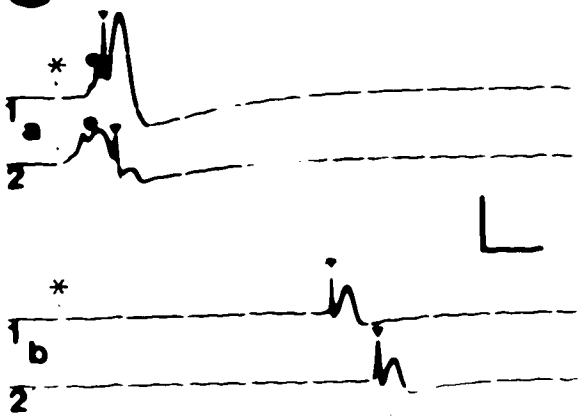
As the form of exumbrellar activated EMPs appeared less complex than the analogous response resulting from subumbrellar stimulation, a comparison of endodermal and ectodermal initiated radial muscle activity was made. When endodermal lamella is stimulated the typical compound EMP/IEP is recorded from radial muscle (Fig. 22a). Activity recorded from the radial muscle during exumbrellar stimulation (Fig. 22b) resembles EMPs recorded from isolated radial muscle (Fig. 10). This suggests that exumbrellar activated crumpling is not associated with endodermal excitation and is confirmed by direct recording from the endodermal lamella (Fig. 23). Exumbrellar stimulation which excites radial muscle usually does not activate endoderm (Fig. 24a). Subumbrellar stimulation elicites IEPs and EMPs with the same recording electrode positioning (Fig. 24b) demonstrating that the failure to record EMPs by IEP in Figure 22a is not due to poor electrode attachment. On rare occasions, endodermal activity was excited by exumbrellar stimulation although this often preceded an initial radial muscle response (Fig. 23c). To resolve this inconsistency, jellyfish were observed for apparent differences in behavior correlated with variation in the degree of excitation resulting from exumbrellar activation. To determine whether this variability is related to the preparation type used whole animals, with out margins isolated, were recorded from. This type of preparation required excessive electrode suction for stable attachment due to the inability to restrain the jellyfish by pinning them flat to the recording dish. Consequently, potentials from whole animals are more complex than the previous recordings from single quadrants of jellyfish. Full crumpling response (i.e. with

FIGURE 22 Variation in crumpling potentials when excited by endodermal or exumbrellar stimulation.

a) Complex crumpling potentials involving RMPs (triangles) and ECPs (circles), recorded from radial muscle upon endodermal lamellar stimulation.

b) Simplified crumpling potentials, involving only RMPs (triangles), recorded at the same positions as in a) upon stimulation of the exumbrellar ectoderm. Symbols as in figure 2. Scale bars - horizontal: 50 m.; vertical: 2 mV.

22



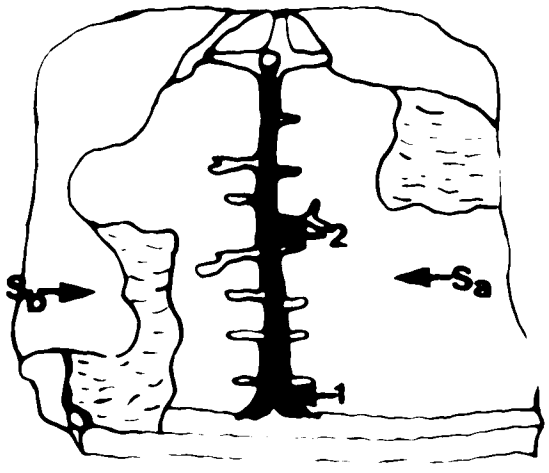
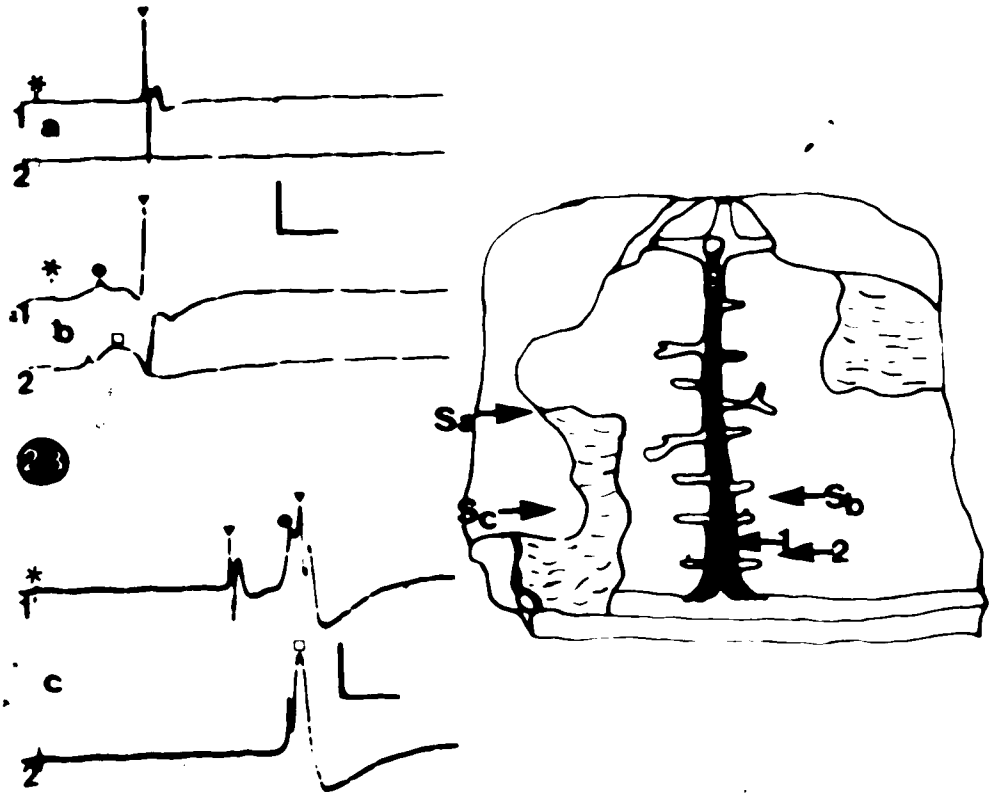


FIGURE 23 RMPs resulting from exumbrellar stimulation without a requirement for endodermal excitation.

a) RMP (triangle) recorded from radial muscle excited by exumbrellar stimulation in the absence of endodermal pulses.

b) Associated ECP (circle) and RMP and endodermal lamellar pulse (ELP; open square) recorded from electrodes as in a) upon endodermal lamellar stimulation.

c) RMP followed by compound RMP/ECP and ELP recorded from electrodes as in a) excited by exumbrellar stimulation. Endoderm can be excited by exumbrellar stimulation although usually after initial radial muscle responses. Symbols as in figure 2. Scale bars - horizontal: a) 100 ms, b) c) 50 ms, vertical: a) b) 2 mV, c) 1 mV.


a
1
2
b
1
2
c
1
2
Sa
Sb
Sc
1
2

sphincter muscle contration) is consistently correlated with excitation of endoderm although endodermal activity usually follows initial RMPs (Fig. 24a). Partial crumpling (i.e. without sphincter muscle contraction) is not, however, associated with endodermal excitation (Fig. 24b). Note that R2 in Figure 24a and b is attached to the subumbrellar surface so that the appearance of ELPs markedly differs from those recorded from endodermal lamella directly (Fig. 2). Simultaneous recordings from two radial muscles during a full crumple shows that although excitation of the radial muscles is generally synchronous, one muscle often twitches first and more frequently (Fig. 24c). Note that the initial RMP of R1 in Figure 23c again precedes endodermal excitation.

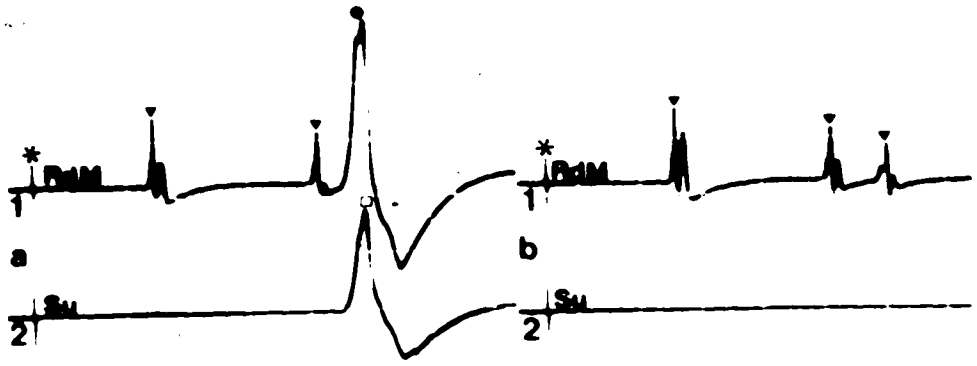
Dividing a radial muscle midway between the margin and apex allows a determination of whether the apical RMP pathway can mediate an exumbrellar stimulated crumple (Fig. 25). Subumbrellar stimulation activates both halves of the muscle as expected (Fig. 25a). However, exumbrellar stimulation of a partial crumpling response only excites that portion of the muscle that is connected to the margin (Fig. 25b). If alternatively endodermal excitation is associated with exumbrellar stimulated radial muscle activity, as during full crumpling, the apical pathway initiates RMPs in that portion of the muscle that is attached to the peduncular base but isolated from the margin (Fig. 25c).

e) Marginal Activity Associated with Crumpling

Spontaneous nervous activity can be readily recorded from the outer nerve-ring (ONR) or the inner nerve-ring (INR) with suction

FIGURE 26 Full and partial crumpling present in a whole animal preparation.

EEP (open square) and ECP (circle) excited by exumbrellar activation of a full crumple a) but not during a partial crumple b) in a whole animal preparation. RMPs (triangles) are excited in both cases and precede endodermal pulses in a). Simultaneous recordings from two radial muscles during full crumpling c) show multiple RMPs and ECPs. Muscle 1 fires before and more frequently than muscle 2. Initial RMP precedes endodermal activity. Symbols as in figure 2. RM - radial muscle; SS - subumbrellar surface. Scale bars - horizontal: a) b) 100 ms, c) 200 ms, vertical: 1 mV.



24

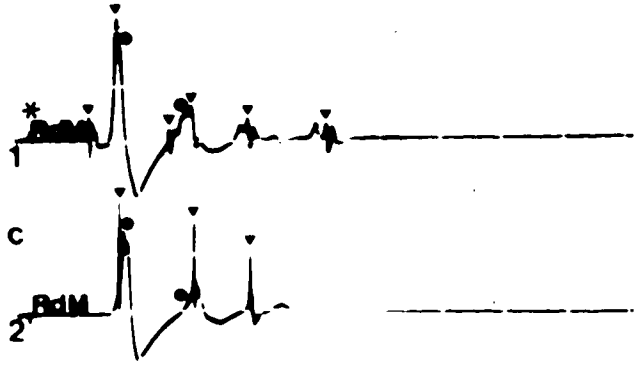
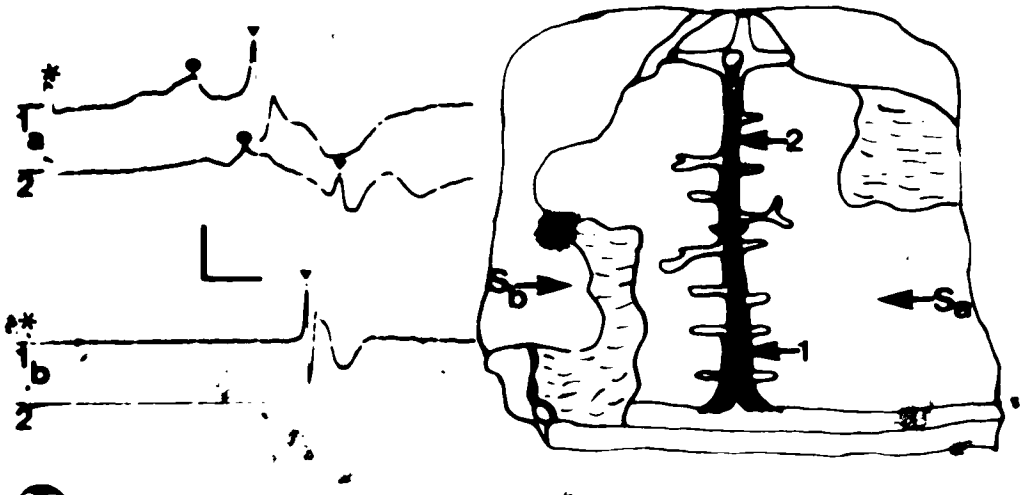
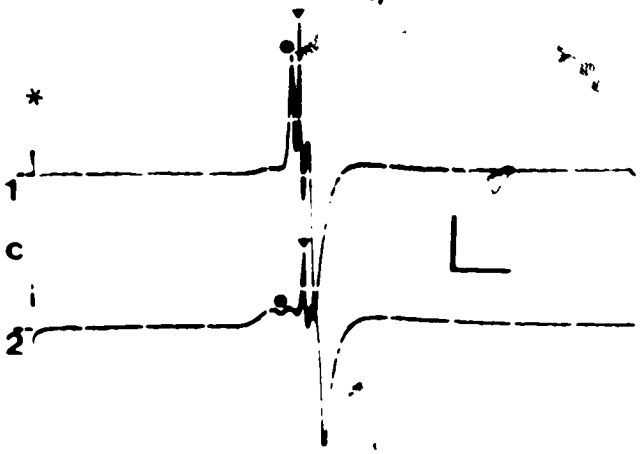


FIGURE 25 RMP pathways active during exumbrellar stimulation of crumpling.

RMPs (triangles) and ECPs (circles) recorded from radial muscle which was cut (semi-circle) midway up the radius. Endodermal lamellar stimulation a) excites both portions of muscle but exumbrellar stimulation of partial crumpling b) does not activate the apical portion of muscle and does not excite ECPs. A similar procedure in a whole animal preparation c) results in an excitation of RMPs and ECPs at both marginal and apical portions of radial muscle when exumbrellar stimulation results in full crumpling. Symbols as in figure 2. Semi-circle marks radial muscle incision. Scale bars - horizontal: a) 20 ms, b) c) 50 ms; vertical: 1 mV.



25



electrodes attached to the base of the velum. Although fine electrode tips (25-40μm) were used it was not possible to discriminate between ONR and INR activity. It was consistently easier to record from the exumbrellar side of the velar base (overlying the ONR) and this was the region recorded from in attempting to correlate nervous events with crumpling.

The usual events recorded spontaneously include pretentacle pulses (PTPs) with associated muscle potential, preswim pulses (PSPs) with associated muscle swim pulses (SPs) and ring pulses (RPs), of cryptic function (Fig. 26), as described by Spencer (1978).

The most typical response of the ma[illegible] full crumpling, to exumbrellar (Fig. [illegible]) subumbrellar (Fig. 27b) stimulation is the excitation of a [illegible] (presumably epithelial) event and 1 to many PTPs (associated with tentacle retraction). Evidence that the large event is endodermal activity is suggested by the fact that movement of the recording electrode towards the ring canal increases the magnitude of the response. Additionally, a large marginal response is seen during a crumple which includes sphincter muscle contraction (Fig. 28a) but not during a partial crumple (Fig. 28b). Note that a series of PTPs is recorded in either case and that the occurrence of endodermal excitation appears at the end of the series (Fig. 28a). The timing of the initiation of PTPs is variable, occurring at times prior to (Fig. 27a) or proceeding (Fig. 27b) endodermal excitation. No nervous activity specific to crumpling was obtained, perhaps due to the likelihood that only a small number of nervous elements would be involved and the probability that these would be masked by the large epithelial potential.

FIGURE 26 Spontaneous outer nerve ring (ONR) activity.

Unrelated traces of spontaneous activity (presumably nervous) recorded from the exumbrellar base of the velum, overlying the outer nerve ring (ONR). Preswim pulses (PSPs; open triangles) and associated swim pulses (SPs; between arrowheads), pretentacle pulses (PTPs; open circles) with associated muscle potential and a ring pulse (RP; closed circle) can be seen. Scale bars -- horizontal: 50 ms, vertical: 0.5 mV.

26

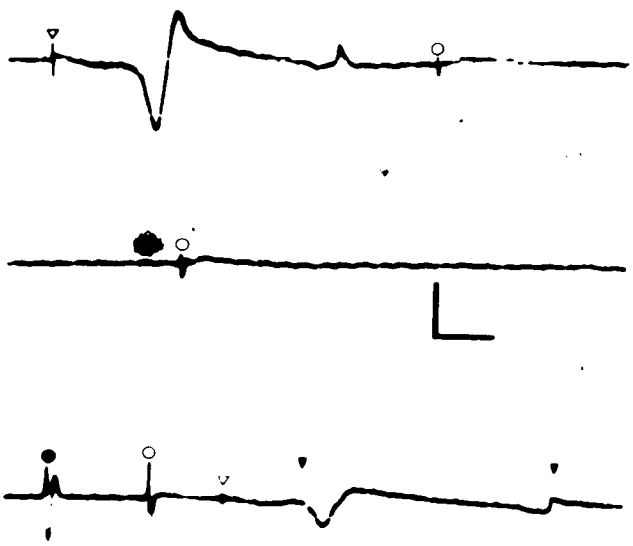
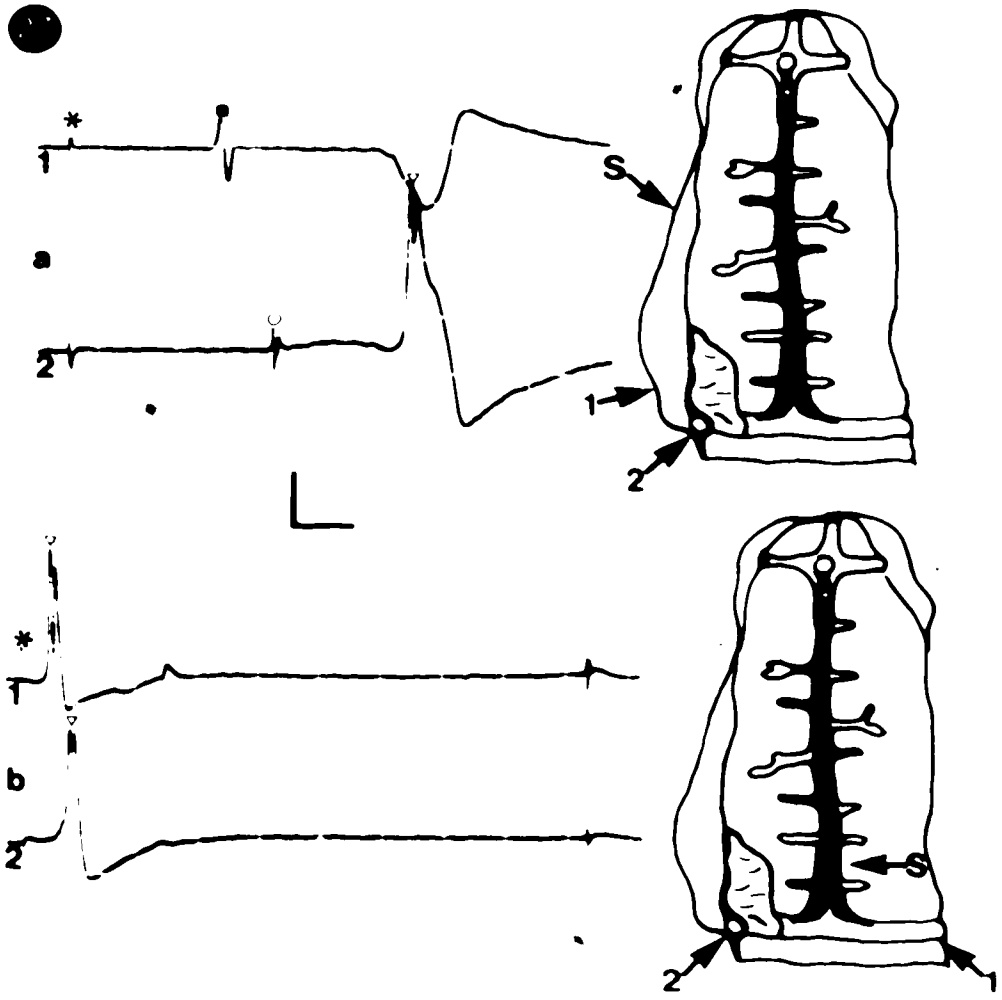


FIGURE 27 Marginal activity excited during crumpling.

a) Stimulation of exumbrella excites an exumbrellar pulse (EP; square) which can activate full crumpling involving excitation of a pretentacle pulse (PTP; open circle) and a large mainly epithelial event (open triangle) recorded from the velar base. Crumpling produced a slow biphasic movement artifact in record 1.

b) Stimulation of endodermal lamella always results in full crumpling also involving large, mainly epithelial events and PTPs when recording from the inner (R1) or outer (R2) base of the velum. Symbols as in figure 2. Scale bars - horizontal: a) 50 ms; b) 100 ms; vertical: 0.5 mV.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2
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b
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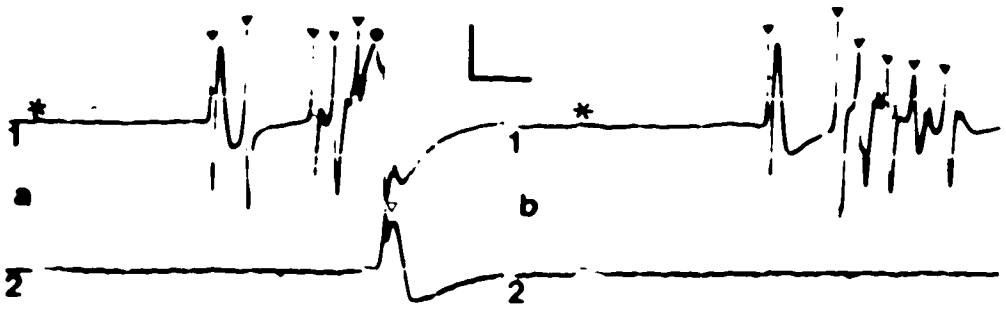
2
1

FIGURE 28 Marginal activity associated with full and partial crumpling.

Series of RTPs (closed triangles) resulting from exumbrellar stimulation associated with an EGP (circle) recorded from radial muscle and a large event (open triangle) recorded from the velar base only during full crumpling a). During partial crumpling b) the RMP train is again excited but no endodermal activity recorded from radial muscle and no large event is recorded from the margin. The large marginal crumpling event in a) is probably mainly endodermal but may include nervous or sphincter muscle activity. Symbols as in figure 2. Scale bars - horizontal: 100 ms; vertical: RT 1.0 mV, RP 0.5 mV.



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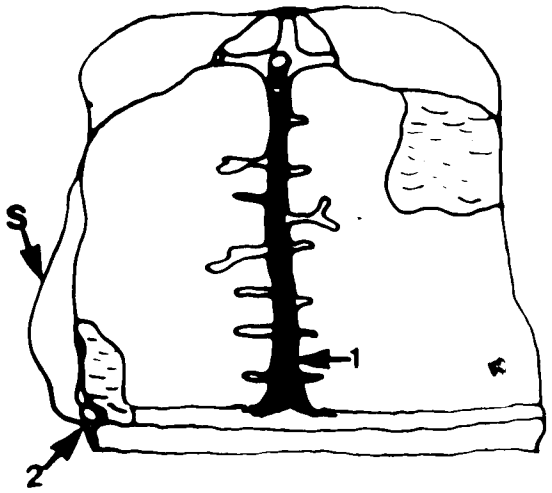


As there is precedence for direct interactions between epithelial and nervous events (Mackie and Passano, 1968; Mackie, 1975), the effect of ectodermal and endodermal stimulation on spontaneous ONR activity was investigated. As previously noted epithelial activity can elicit tentacle retraction. This is associated with an initia[illegible] of PTPs which may occur as a train of numerous potentials follow[illegible] epithelial excitation (Fig. 29a). In addition, there is some ev[illegible]e for an inhibition of swimming pulses (SPs) consequent to crumpli[illegible] (Fig. 29b). This relationship is complicated by the fact that a spontaneous train of PTPs often sets off swimming after which the PTPs cease (Fig. 39c). To make more definitive statements about the precise interrelationships between epithelial excitation during crumpling and nervous activity will require a quantitative analysis which takes into consideration inherent activity cycles.

f) Structural Aspects of the Radial Muscle Excitation Pathway

(i) Ultrastructural features of radial muscle

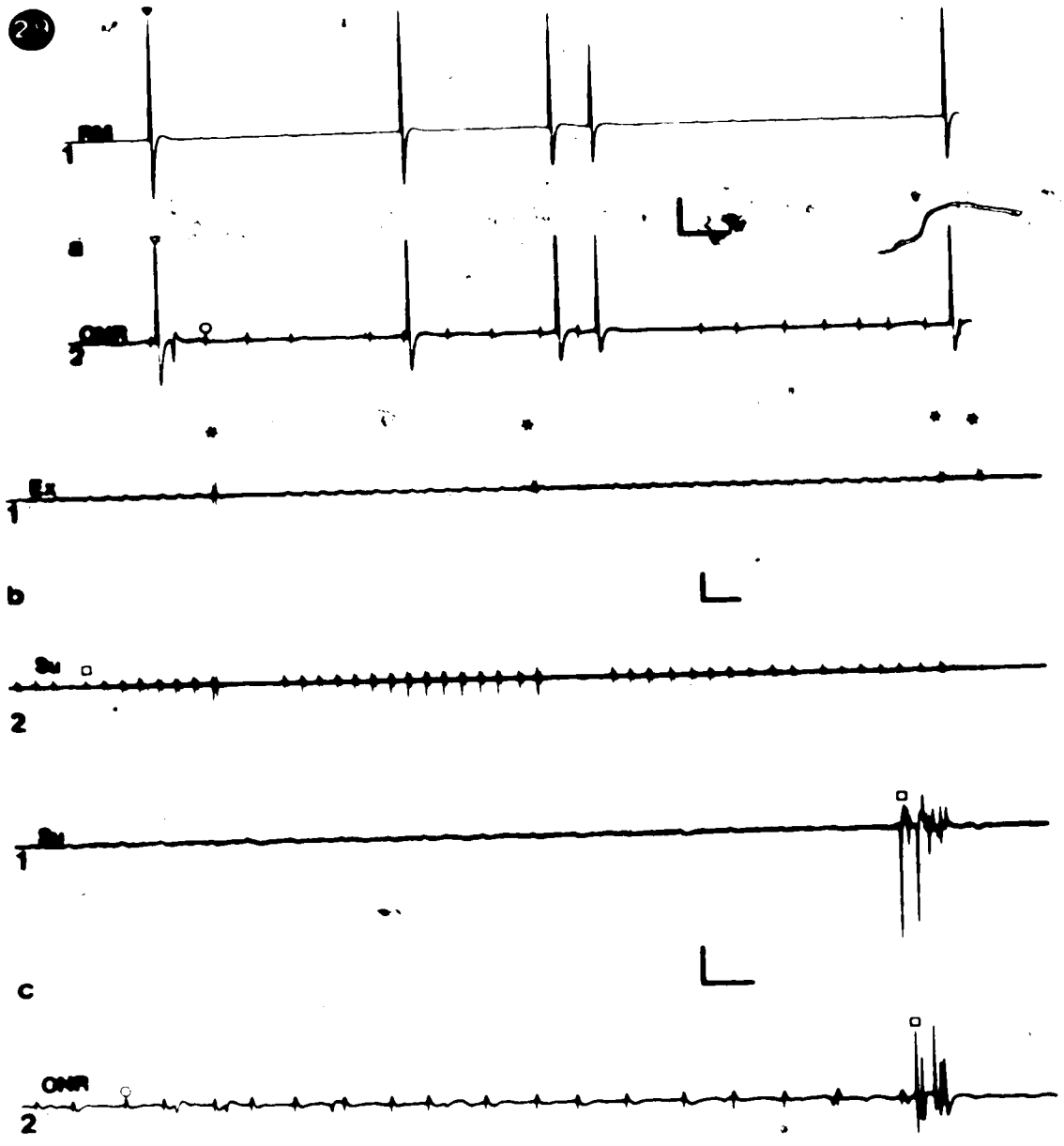
Radial muscle in *Polyorchis penicillatus* occurs as a well-developed bundle of smooth myoepithelium containing thick and thin myofilaments (Plate 10). The internal surface area of radial muscle is greatly increased by the presence of mesogleal invaginations which pass almost to the subumbrellar surface (Plates 1a; 10a). Evidence for gap-junctions has not been established although septate junctions are numerous at the subumbrellar surface. The most common specialized intercellular contacts between radial myocytes are "desmosome-like" junctions, present at their basal, myofibril-containing ends (Plate 10b, c). These junctions are characterized by the presence

FIGURE 29 The effect of crumpling on marginal activity.

a) Train of PTPs (open circles) and marginal events (open triangles) associated with crumpling indicated by RMPs (closed triangles) with stimulation of endodermal lamella. Each RMP marks a separate stimulus.

b) Inhibition of a spontaneous cycle of swimming pulses (SPs; open squares) recorded from subumbrellar swimming muscle by stimulation of the exumbrellar ectoderm (asterisk). EPs are excited with each stimulus recorded by R1.

c) Spontaneous series of PTPs recorded from ONR setting off a burst of swimming recorded from the subumbrellar surface and ONR. Symbols: RM - radial muscle, ONR - outer nerve ring; Ex - exumbrellar surface; Su- subumbrellar surface. Scale bars - horizontal: 2s; vertical: a) R1 0.5 mV, R2 0.2 mV, b) 0.1 mV, c) 0.2 mV.


a
b
c

of membrane densification and filaments passing between apposed cell membranes at various points (Plate 10c). In addition, radial myocytes contain microtubules in small aggregates, parallel to the myofilaments, at the basal ends of the cells (Plate 10c).

Epithelial connections between basal processes of radial muscle and radial canal (Plate 11a, c) are numerous throughout the length of the perradius. It appears that these connections include myofibrils (Plate 11b). Nerves often occur in the mesoglea between radial muscle and canal often in conjunction with these epithelial bridges (Plates 11a, c). Although the exact source of these nerves is unclear, it may be suggested that they connect the radial nerves and the peripheral radial canal nerves (Plate 1b, c). Note the presence of nerve distal from the radial nerve bundle in Plate 12a.

(ii) Radial nerve synapses

The radial nerve bundles occur at the lateral bases of the radial muscle (Plate 1a) apposed to both radial muscle and subumbrellar swimming muscle (Plate 12a). Gap-junctions (Plate 12b) and asymmetrical synapses (Plate 12c, d) are common between radial neurites. Synapses between radial nerve and apical extensions of the subumbrellar swimming myoepithelium occur but are infrequent (Plate 12a, 13). These neuro-myoepithelial junctions display pre- and postsynaptic membrane densification and presynaptic vesicles (usually clear-cored). These vesicles are of various diameters even within the same junction (see especially Plate 13b). The most numerous synapse of radial nerve is with radial myoepithelium. These junctions also show membrane densification and presynaptic

vesicles which are either clear-cored (Plate 14a, d) or dense-cored (Plate 14b, c, d).

(iii) Ultrastructure of the interradial margin

Sphincter muscle has many structural features analogous to radial muscle. They are both smooth myoepithelia with mesogleal infoldings (Plate 10a, 15a) and have numerous "desmosome-like" intercellular junctions and scattered small arrays of microtubules (Plate 10c, 15b). In addition, both muscles have a similar histological relationship with endodermal canal (Plate 1a, 2a) and epithelial connections between sphincter muscle and ring canal are common as in the radius (Plate 11a, c). Extending the comparison further both sphincter and radial muscle are closely associated with nerves, there being an extensive apposition of sphincter muscle and outer nerve ring (ONR). As with radial nerve, the ONR has numerous interneuronal synapses (asymmetrical, Plate 15c and symmetrical, Plate 15d) and neuromyoepithelial junctions with smooth muscle (Plate 16). These synapses between ONR neurites and sphincter muscle (Plate 16) appear to be restricted to or at least most common at the "triradius" along the face of mesoglea passing to the velum.

(iv) Connections of radial muscle to the margin

Radial sections through the margin at the perradius pass longitudinally through the radial muscle and radial canal (Plate 17). Notable features of this region of the margin are the great reduction in height of the sphincter muscle (cf. Plate 17a and Plate 2a) and

muscular and nervous connection across the velar mesoglea from the subumbrellar to the exumbrellar side of the margin (Plate 17b, c, d, e, f). At the electron microscope level, the reduction of sphincter muscle is especially evident (cf. Plate 18 and Plate 15) which appears to be restricted to long narrow processes extending almost to the exumbrellar surface. In addition, there is exumbrellar smooth muscle along the velar mesogleal face although the presence of radial muscle bridges between subumbrella and exumbrella (Plate 17c, d, e, f; 19) makes it uncertain whether the source of this muscle is sphincter or radial muscle. Distinction between these two muscles at the perradial margin is probably artificial in that there appears to be a physical connection between them. Attachments between radial muscle processes and ring canal are also common at the perradius (Plate 17b, d).

Synapses between nerve and smooth muscle are very common at the perradial margin. Synapses between neurites of the inner nerve-ring (INR) and radial muscle have been located (Plate 20) although they are not frequent. The more common synapse is between outer nerve-ring (ONR) neurites and smooth (radial and/or sphincter) muscle (Plate 21). Note that the exact location of these five synapses is indicated in Plate 18. As with radial nerve-radial muscle synapses (Plate 14) these junctions show pre- and postsynaptic densification and presynaptic vesicles with clear or dense cores. These ONR neuromuscular junctions are almost always located along the velar mesogleal face in the vicinity of which radial muscle bridges from the subumbrella have been observed (Plates 17, 19).

DISCUSSION

I. EPITHELIAL CONDUCTION

The demonstration of excitable epithelia mediating behavioral patterns in a diverse assemblage of animals is an example of a scientific advance which necessitates a re-evaluation in ideology. Even with the general acceptance that the rapid reception, conduction and integration of information and consequent initiation of a reaction is the property of the nervous system researchers often speculated that epithelia may provide an additional substrate for the propagation of activity. Kleinenberg (1872) suggested that the epitheliomuscular cells of *Hydra* function as independent effectors, in which apical sensory excitation directly activates the basal muscular processes of the same cell. Chun (1897) expanded on this theory by proposing that there may be a conduction of excitation between epitheliomuscular cells and even between the simple epithelial cells of the siphonophore exumbrella where no muscular processes are found. Wintrebert (1904) speculated that the skin of frog tadpoles had an "irritabilité" which provided the tadpoles with an ability to respond to external stimuli. In his monograph on the primitive nervous system, Parker (1919) suggested that "neuroid conduction" may exist within the tissues of sponges and between ciliated epithelial cells. These reports were largely ignored at the time due to a scarcity of experimental evidence and the concentration of neurobiologists' efforts to resolve the structural and functional basis of nervous conduction. Since the work of DuBois-Reymond (1848) it was acknowledged that nervous

activity involves the flow of electric current. With this understanding, two theories were advanced to explain the anatomical basis of nervous conduction. One group believed in the "reticularism" of the nervous system; that there exists a complete protoplasmic continuity throughout the system. Other biologists insisted that the nervous system must be composed of discrete units, neurons, requiring that some form of transfer between cells must exist for nervous propagation. This latter "neuron doctrine" eventually became established in the early 1900's largely due to the innovative work of Ramon y Cajal (reviewed by Cajal, 1954), who perfected techniques to visualize individual neurons, and to Sir Charles Sherrington (1906), who originated the term "synapse" in his lucid speculations that transmission between neural elements differs from the conduction of activity along a neuron and that this transmission can be excitatory or inhibitory. It was generally considered that nervous conduction must involve a direct electrical transfer between neurons to meet the established speed of conduction. However, chemical transmission by nerves was suggested by Elliot (1904) and Loewi (1921), demonstrated by Dale, Feldberg and Vogt (1936) and finally confirmed to exist at the neuromuscular junction (Fatt and Katz, 1952) and between motoneurons (Brock *et al.*, 1952). There now exists a wealth of evidence that chemical transmission between nerves and between nerve and effector occurs in many animals, and that the site of this transmission is the synapse (reviewed by Elfvin, 1976). The first evidence that the mechanism of excitation spread is not entirely standardized came with the demonstration of electrical transmission between

a neuron of the abdominal nerve cord and a motoneuron in the crayfish (Furshpan and Potter, 1959). This proved to be an impetus which resulted in the demonstration of electrical connections between nerves in a variety of invertebrate and vertebrates systems (reviewed by Bennett, 1972, 1974; Sotelo, 1977). The proof that the conduction of excitation is not restricted to the nervous system came when Mackie (1965) presented thorough histological evidence for the absence of nerves and contractile elements from an impulse propagating epithelium in a siphonophore. Since this report intracellular recordings have verified the excitability of mammalian pancreatic islet β-cells (Dean and Matthews, 1968; Matthews and Sakamoto, 1975), the skin of the amphibian tadpole (Roberts, 1969; Roberts and Stirling, 1971), mammalian adrenocortical cells (Matthews and Saffran, 1973), the skin of an ascidian tadpole (Mackie and Bone, 1976), endoderm (Mackie, 1976b; Spencer, 1978) and ectoderm (Josephson and Schwab, in preparation) of hydrozoans, gastropod salivary gland cells (Kater *et al.*, 1978b) and photocytes within the elytra of a polychaete (Herrera, 1979). In order to propagate excitation, epithelial conduction requires that the electrical current associated with the action potential in one cell be passively spread, without severe attenuation, to adjacent cells. We thus have systems which invoke the original explanation for the conduction of excitation. With the understanding that numerous excitable epithelia exist it can no longer be assumed that nervous conduction fulfills the only means of rapid communication in any system.

a) Physiology of Epithelial Conduction

The physiological analysis of epithelial conduction has shown that there is a convergence of function despite the diversity of systems involved. Three basic functions of excitable epithelia have been elucidated.

(i) Initiation of muscular activity for protection or escape

Prior to the demonstration that siphophores have excitable epithelia (Mackie, 1965), Mackie (1964) suggested that the reverse swimming response of the physonectid siphonophore *Nanomia* [redacted] activated by conduction of excitability from the exumbrellar ectoderm. Siphonophores are colonial and polymorphic hydrozoans with medusoid and polypoid individuals. Reverse swimming involves the concerted contraction of the circular (swimming) muscles of all medusoid individuals (nectophores) and simultaneous contraction of the "fibers of Claus", which are radial fibers attached to both sides of the velum. The contraction of these latter fibers orients the bell opening of the nectophores up so that the water jet leaving the bell forces the colony down. This response is initiated by stimulation of the upper nectophores. Although there has been no verification of excitability of the ectoderm in *Nanomia*, this can be inferred from the studies of closely related siphonophores (Mackie, 1965).

Epithelial conduction in the calycophoran siphophore, *Hippopodius* has been more thoroughly examined (Mackie, 1965, 1976b; Mackie and Mackie, 1967; Bassot *et al.*, 1978). The most obvious response to stimulation of the excitable exumbrella of a nectophore

is the involution of the margin due to the contraction of radial muscle of the velum (ectodermal) and of the pseudovelum (endodermal). It has been proposed that this response serves to protect the marginal nerves (Mackie and Mackie, 1967). Some other effector responses are associated with epithelial activity in *Hippopodius* and will be discussed in the next sections.

Many solitary hydrozoan medusae display an analogous behavior to the involution of *Hippopodius* known as crumpling. Crumpling and its association with epithelial conduction has been reported in the anthomedusae, *Tiarella*, *Euphysa* (Mackie and Passano, 1968), *Sarsia* (Mackie and Singla, 1975; Mackie, 1975) and *Polyorchis* (Spencer 1978), in the leptomedusan *Phialidium* (Mackie and Passano, 1968) and in the limnomedusan *Proboscidactyla* (Spencer, 1975).

The first epithelial system associated with an escape response amenable to intracellular recording techniques was the skin of the tadpoles of the amphibians *Xenopus* (Roberts, 1969; Roberts and Stirling, 1971), *Rana* and *Rana* (Roberts, 1971). Behavioral and cutting experiments (Roberts, 1971) have shown that skin impulses when excited by tactile or electrical stimulation can activate swimming provided that some epithelial cells are connected to the sensory system (via the Rohon-Beard cells). This excitability provides the tadpoles with a precocious ability to respond to external stimuli before a complete sensory innervation of the skin develops.

There have also been recent reports of excitable skin in all three classes of the Urochordata. Bone and Mackie (1975) showed

with extracellular recordings that the outer epithelial surface of the tail and trunk of the larvacean *Oikopleura* is excitable. Excitation of the skin results in a stimulation or an acceleration of the swimming rhythm presumably through the conduction of impulses to the caudal ganglion via the sensory Langerhans nerves. Intracellular recordings have confirmed the skin excitability of the larva of the ascidian *Dendrodoa* (Mackie and Bone, 1976). The skin impulses in this tunicate, contrary to that in *Oikopleura*, inhibit unilateral and symmetrical flexions of the tail. The significance of this behavior to the tadpole is unclear unless these animals are negatively buoyant and constantly active, in which case an inhibition of swimming may be the only means of avoiding an obstacle or aggressor. Finally, the ectoderm of the test, the ventral and lateral regions of pharyngeal endoderm and the epithelium between the peripharyngeal bands and inhalent siphon are excitable in the blastozooids of several colonial thaliaceans (Mackie and Bone, 1977). The function of epithelial conduction in these tunicates is complicated by the fact that all changes in locomotion (acceleration, inhibition and reversal) are apparently associated with the spread of skin impulses.

(ii) Activation of glandular secretion

The first demonstration of an excitation of action potentials in a glandular epithelium involved the mammalian β pancreatic-islet cells (Dean and Matthews, 1968; Matthews and Sakamoto, 1975). The results from a number of studies have shown that this is a prime model for analysis of the mechanisms underlying excitation-secretion coupling (reviewed by Matthews, 1977). Action potentials in the pancreatic cells

can be induced by high glucose concentrations, with the frequency of spike discharge being directly proportional to glucose concentration, or by depolarizing current injection (Matthews and Sakamoto, 1975). The ionic basis of this action potential appears to be Ca^{++} influx as demonstrated by the inability of high glucose levels or current injection to excite pancreatic cells in a Ca-free medium (Matthews and Sakamoto, 1975). In addition, the effectiveness of glucose analogues in exciting the cells is dependent on the ability of the pancreatic cells to metabolize them (Dean *et al.*, 1975). Thus it is apparent that the glucose-dependent electrogenesis of β pancreatic-islet cells provides a mechanism for stimulating the release of insulin through the gated influx of Ca^{++} and subsequent binding of insulin-containing granules to the plasma membrane leading to their exocytosis. Although the propagation of action potentials between pancreatic islet cells has not been investigated (Matthews, personal communication), there is evidence that the pancreatic cells are electrically coupled (Meissner, 1976). Consequently, it is possible that the excitability of this epithelium serves to conduct the secretory stimulus between cells as well as to induce the secretion of individual cells.

It has also been reported that mammalian adrenocortical cells, when bathed in a K^+-free medium, exhibit regenerative action potentials when exposed to adrenocorticotrophic hormone (ACTH) (Matthews and Saffran, 1973). This excitability may be indicative of the stimulation of steroid production in the presence of ACTH but the requirement for unnaturally low K^+ levels makes the significance of this response to the intact animal unclear.

Another function of epithelial conduction in the siphonophore *Hippopodius* was provided with the demonstration of secretion by the endodermal *rete mirabile* correlated with excitability (Mackie, 1976b). This was also the first verification of epithelial excitability and electrical coupling in coelenterates through the use of intracellular electrodes. As it is known that exumbrellar simulation excites the subumbrellar endoderm (Bassot *et al.*, 1978) it is conceivable that this secretion is a defense mechanism although determination of the secretory product and behavioral studies would be required to establish this.

Finally, evidence has been provided for the excitability of the salivary glands of several gastropods (Kater *et al.*, 1978b). The gland cells are connected by low-resistance pathways which allow for propagation of a regenerative, non-decrementing action potential. There is evidence that this excitability is correlated with secretion (Kater, personal communication). Of particular significance is the innervation of the salivary glands by identified buccal ganglion neurons which integrates the excitation of the salivary glands with the feeding cycle (Kater *et al.*, 1978a).

(iii) Activation of bioluminescence and blanching

The first report of epithelial conduction (Mackie, 1965) mentioned that impulse propagation is associated with luminescent flashes and reversible "blanching" of the nectophores of *Hippopodius* in addition to the involution of the margin (discussed previously). "Blanching" refers to the gradual development of opacity of the normally transparent bell due to the production of mesogleal granules

and requires an intact epithelial covering for conduction of the response and for deblanching (Mackie and Mackie, 1967). The full development of the response requires up to 10s and shows no facilitation, with the first shock being most effective and each subsequent shock producing less of a response (Bassot *et al.*, 1978). Bioluminescence, when excited by a single stimulus occurs as a single flash (40-400ms) or series of flashes and repetitive stimulation causes a facilitation in intensity of the bioluminescent response (Bassot *et al.*, 1978). The site of luminescent emission in *Hippopodius* appears to be the exumbrellar ectoderm (Bassot *et al.*, 1978). *Hippopodius* then has four distinct responses (involution, endodermal secretion, blanching and luminescence) associated with epithelial conduction. Although not proven it is assumed that they serve a common protective function, excited by an agitation of the exumbrella of any nectophore.

A recent study, using the dorsal epithelium of the elytra (scales) of the polynoid polychaete *Harpe[illegible]* (Herrera, 1979), has provided the first conclusive evidence for the excitation-luminescence coupling of an epithelium with intracellular recording techniques. Ionic studies of the scale photocytes have demonstrated the conduction of Na^{+}-dependent spikes between photocytes and a direct correlation between excitation of a Ca^{++}-dependent spike and a luminescent flash (Herrera, 1979). This study has also shown a synaptic interaction between segmental nerves and the photocytes which elicits a Ca spike in the photocytes and luminescent emission. Mechanical stimulation of the dorsal epithelium of the worm excites

bioluminescence either through an epithelial pathway or through the stimulation of sensory nerves and subsequent segmental nerve activity.

b) Structural Basis of Epithelial Conduction

(i) Gap-junctions

From the physiological evidence that intercellular ionic coupling occurs in epithelia (Loewenstein and Kanno, 1964; Lowenstein *et al.*, 1965; Penn, 1966; Loewenstein and Penn, 1967; Jamakosmanovic and Loewenstein, 1968; Popowich and Caveney, 1976; Meissner, 1976; Mackie, 1976b; Kater *et al.*, 1978b) it has been assumed that coupling is a general characteristic of epithelia. Note that only the latter three studies have involved excitable epithelia where the function of the coupling is obvious. In inexcitable epithelia the demonstration of electrical coupling is assumed to be significant only in demonstrating the presence of low-resistance, ionic pathways between cells. It has been assumed that this intercellular connection provides a means for the transport of metabolites between inexcitable epithelial cells and between embryonic cells (reviewed by Sheridan, 1974; Staehelin, 1974; Gilula, 1977). Evidence for this is, however, circumstantial since it is based on studies of tissues in culture (reviewed by Sheridan, 1976; Pitts, 1977). The precise morphological substrate of electrical coupling in invertebrate epithelia is in doubt due to the frequent coexistence of septate and gap-junctions (Hagopian, 1970; Flower, 1971; Gilula and Satir, 1971; Hudspeth and Revel, 1971; Noirot and Noirot-Timothée, 1971; Rose, 1971; Hand and Gobel, 1972; Johnson *et al.*, 1973). Nevertheless, it can be inferred that gap-junctions are the

sites of coupling in these tissues considering the results of studies on analogous systems. A direct correlation has been established between electrical uncoupling and fine structural disarrangement of gap-junctions in vertebrate cardiac muscle (Barr *et al.*, 1965), crayfish lateral giant axon (Asada and Bennett, 1971; Pappas *et al.*, 1971; Peracchia and Dulhunty, 1976) and mammalian stomach and liver (Peracchia, 1977). Coupled systems have also been described in which gap-junctions are the only intercellular contact found (Dreifuss *et al.*, 1966; Gilula *et al.*, 1972; Pinto da Silva and Gilula, 1972; Rash and Fambrough, 1973; Azarnia *et al.*, 1974; Oliveira-Castro *et al.*, 1975).

It has been assumed that epithelial conduction is mediated through gap-junctions based on the reported presence of close membrane appositions in conventionally fixed and stained material (Roberts and Sirling, 1971; Joseph *et al.*, 1973; Bone and Mackie, 1975; Mackie and Singla, 1975; Mackie, 1976b; Mackie and Bone, 1976, 1977; Bassot *et al.*, 1978). In *Hydra* there is good evidence for epithelial gap-junctions (Hand and Gobel, 1972; Wood, 1977) and indirect evidence for epithelial conduction (Campbell *et al.*, 1976). This latter evidence is based on studies of nerve-free (colchicine-treated) *Hydra* in which no spontaneous activity can be recorded although strong mechanical stimulation produces contraction pulses resulting in column shortening. There is a report of intracellularly recorded epithelial potentials in *Hydra* (Kass-Simon and Diesl, 1977) but no firm evidence for epithelial excitability is presented. This study involved recordings from dissociated epithelial cells and the

very low and variable values given for resting potentials (+3 to +25mV for endoderm; -2 to -25mV for ectoderm) suggests an unhealthy condition of the cells or an incomplete penetration. In addition, the potentials reported are largely membrane oscillations or "junctional-like" potentials and it is stated that this activity may be the result of attached nerves and not endogenous to the epithelial cells. The only previous studies, that I am aware of, which clearly correlate the presence of specialized junctions with excitability in a epithelium are those of Orci, Unger and Renold (1973) and Matthews and Sakamoto (1975).

In sectioned material, the gap-junctions of *Polyorchis* correspond in detail with those reported in a variety of invertebrate and vertebrate tissues (reviewed by McNutt and Weinstein, 1973; Staehelin, 1974; Gilula, 1977), with the possible exception of the larger intercellular gap (4-5nm). This is considerably greater than the 2-3nm gap of vertebrate tissue (Brightman and Reese, 1969; Goodenough and Revel, 1970; McNutt and Weinstein, 1970), more closely resembling the 3-4nm gap generally found in arthropod tissues (Hudspeth and Revel, 1971; Pappas *et al.*, 1971; Rose, 1971).

That the globule-bearing plaques represent *en face* views of gap-junctions is directly verified by the comparable widths and periodicities of these globules and the electron-lucent bands seen in transversely sectioned gap-junctions. In addition, the central electron-opaque line within some of the lucent globules of transversely sectioned gap-junctions probably corresponds to the central opaque dot of *en face* globules. The apparent entrance of lanthanum into

the globules is enigmatic in that it suggests an incomplete insulation of the intercellular bridge. This does, however, give physical evidence for the existence of a channel which could fulfill ionic coupling.

The freeze-fracture replica particle aggregates of *Polyorchis* show a close correspondence with the *en face* lanthanum filled gap-junctions. This technique demonstrates the adhesion of gap-junction membrane particles to the outer, exoplasmic face (EF) as described in arthropods (Flower, 1972; Satir and Gilula, 1973; Johnson *et al.*, 1973; Dallai, 1975), in *Hydra* (Filshie and Flower, 1977; Flower, 1977; Wood, 1977), in a platyhelminth (Flower, 1977) and in annelids (Flower, 1977; Larsen, 1977). This is the opposite polarity of vertebrate (Goodenough and Revel, 1970; Larsen, 1977) and molluscan (Flower, 1971; Gilula and Satir, 1971) gap-junctions were the particles adhere to the inner, protoplasmic face (PF). It is not known whether this difference in fracturing characteristics is an intrinsic property of the different gap-junctions or is a property of the general plasma membrane.

It has been firmly established that the globules within gap-junctions are typically closely packed and hexagonally arranged with periodicities between 8-10nm, in vertebrate (Revel and Karnovsky, 1967; Brightman and Reese, 1969; Goodenough and Revel, 1970; McNutt and Weinstein, 1970; Zampighi and Robertson, 1973; Albertini and Anderson, 1974; Keeter *et al.*, 1975; Caspar *et al.*, 1977) and invertebrate (Pappas *et al.*, 1971; Lorber and Rayns, 1972; Hand and Gobel, 1972) tissues. Alternatively, some studies have reported

more variable globule arrangements with periodicities as high as 20nm in invertebrate tissues (Hudspeth and Revel, 1971; Johnson *et al.*, 1973; Peracchia, 1973). This ambiguity has been resolved by analysis of gap-junctions before and after uncoupling treatments in crayfish nerve (Peracchia and Dulhunty, 1976) and in mammalian stomach and liver (Peracchia, 1977). These studies have clearly shown that loosely and irregularly packed globules (with a periodicity of 20nm for the nerve and 9.5-13.5nm for stomach and liver) of coupled tissue become tightly and hexagonally arranged (with a periodicity of 15nm for the nerve and 8.5nm for stomach and liver) when uncoupled. Furthermore, the gap of transversely sectioned material is reduced from 4-5nm to 2-3nm by uncoupling proceedures in the crayfish lateral nerve (Peracchia and Dulhunty, 1976). Although analysis of gap-junctions before and after uncoupling has not been performed in *Polyorchis*, it can be speculated that the larger intercellular gap (4-5nm) and non-hexagonal arrangement of globules is indicative of a functional condition of the gap-junctions at the time of fixation.

Within the endodermal canals of *Polyorchis* the gap-junctions are concentrated at the basal end of the endodermal cells (ca. 35-40μm distant from the septate junctions). This localization is unlike that of many invertebrate epithelia where there is a close association of gap and septate junctions (Flower, 1971; Gilula and Satir, 1971; Hudspeth and Revel, 1971). The peripheral location of gap-junctions in *Polyorchis* may be important in removal of the site of ionic coupling from the external saline within the canal lumen.

(ii) Septate junctions

Since the initial description by Wood (1959) in *Hydra*, septate junctions have been reported in a variety of invertebrate phyla (reviewed by Staehelin, 1974). In transverse section the septate junctions of *Polyorchis* and other invertebrates are comparable. However, in tangential section the smooth, parallel contour of the septa in *Polyorchis* indicates that these junctions are of the "Hydra type" (Danilova *et al.*, 1969; Staehelin, 1974). This type of septate junction is apparently restricted to the Cnidaria since it has only been reported in *Hydra* (Hand and Gobel, 1972; Filshie and Flower, 1977), *Pelmatohydra* (Danilova *et al.*, 1969) and *Phialidium* (Leik and Kelly, 1970). In many other phyla, including the Platyhelminthes (Storch and Welsh, 1977), Annelida (Baskin, 1976), Arthropoda (Locke, 1965; Danilova *et al.*, 1969; Rose, 1971; Noirot-Timothée and Noirot, 1973) and Mollusca (Gilula *et al.*, 1970; Flower, 1971), a second type of septate junction (pleated sheet or honeycomb) has been reported.

Two functions have generally been attributed to septate junctions. Firstly, they undoubtedly serve as an adhesion point between cells. As evidence for this function Bibb and Campbell (1973) have shown that epidermal healing in *Hydra* begins with the formation of septate junctions between apposed cells. Secondly, evidence has been presented to suggest that septate junctions act as pericellular permeability barriers. Loewenstein and Kanno (1964) and Josephson and Macklin (1969) showed electrophysiologically the presence of a high resistance barrier between the exterior and lumen of the insect salivary gland

and between the exterior and gastrovascular cavity of *Hydra*, respectively. Septate junctions occur at the lumen of the salivary gland (Wiener *et al.*, 1964) and at the ectodermal surface and endodermal lumen of *Hydra* (Wood, 1959; Hand and Gobel, 1972) and this at least suggest their involvement in establishing a transverse permeability barrier. In addition, Szollosi and Marcaillou (1977) have correlated the existence of septate junctions in the basal compartment of the locust testicular follicle with the absence of lanthanum and peroxidase penetration into this layer. Furthermore they demonstrated that the apical compartment is freely permeable to these tracers and contains no septate junctions. Perhaps the most direct evidence for considering these junctions to be permeability barriers comes from the study of Lord and DiBona (1976) in which it was clearly demonstrated that the septate junctions of planaria are functionally analogous to vertebrate tight junctions. It has been shown that the usual resistance to water and ionic movement from the mucosal (outer) surface to the serosal (inner) surface in amphibian epithelia by tight junctions is decreased upon a reversal of the osmotic gradient so that inward movement of water is favored (Urakabe *et al.*, 1970; DiBona, 1972). This osmotically induced conductance is provided by the formation of fluid filled "blisters" at the tight junctions (DiBona and Civan, 1971; Wade and Karnovsky, 1974). Exposure of planaria to hypertonic solutions produced a similar blistering of the intercellular space of the septate junctions (Lord and DiBona, 1976).

In *Polyorchis*, septate junctions are limited to the lumenal border in the endodermal canals and to the exumbrellar and

subumbrellar surfaces in ectoderm. These junctions extend only short distances (ca. 3-4μm) yet encompass numerous cellular interdigitations as described in other hydrozoans (Overton, 1963; Leik and Kelly, 1970; Hand and Govel, 1972; Wood, 1977). Freeze-fracture replicas demonstrate that although the septate junctions only extend short distances, the rows of septa can occupy considerable lateral depths and are often convoluted. The presence of interdigitations and the arrangement of septa in the plane of the cell surface probably increase the diffusional resistance between the endodermal lumen or between the ectodermal surface and the mesogleal face of the tissue by imposing additional complexity to the extracellular space.

Although the endodermal septate junctions of *Polyorchis* are minimally impregnated with lanthanum, they provide little restriction to penetration by the tracer as demonstrated by the thorough saturation of gap-junctions. Because the endodermal canals are surrounded by mesoglea, lanthanum can only reach gap-junctions via the canal lumen and septate junctions. It is possible that this free permeability is a consequence of fixation. This is supported by the fact that other workers have reported an absence of (Szollosi and Marcaillou, 1977) or a minimal (Hand and Gobel , 1972; Filshie and Flower, 1977) penetration through septate junctions when lanthanum is added to living tissue.

In constrast to the endodermal canals, the insulating ability of septate junctions in the endodermal lamella is likely to be of lesser importance since this tissue is presumbaly isolated from the

surrounding seawater by the mesoglea. I suggest that septate junctions in the endodermal lamella function mainly to maintain tissue integrity during displacement of the lamella as occurs with the contraction of the swimming, radial and sphincter muscles.

(iii) Proposed synergy of gap and septate junctions in establishing epithelial excitability

For a tissue to be excitable there must be some means of relaying the regenerative action potential of one cell to adjacent cells without attenuation. Nerves are composed of neurons with long axonal processes which are specialized to conduct impulses, often over long distances, through a passive ionic current spread which regenerates an action potential before attenuating. At the neuronal terminal of most nerves the impulse elicits release of a chemical transmitter, by fusion of synaptic vesicles with the plasma membrane, which combines with receptors to excite the postsynaptic nerve or effector. In an epithelium, which shows nonpolarized transmission, the passive current flow associated with the action potential of one cell must produce excitation of surrounding cells. To effect this transfer of excitation the core conducting properties of an epithelial cell must not be severely reduced at the intercellular gap. Thus it is clear that there must be low-resistance ionic connections between cells. Because the current flowing into an unexcited cell must pass through the plasma membrane to the external conducting medium in order to depolarize the cell to impulse threshold, there must be a considerable resistance between the cytoplasm and the conducting medium to

limit shunting of the current. It is, therefore, suggested that the gap-junctions provide the channels for an insulated current flow between cells and that the septate junctions increase the resistance between the current pathway and the external saline medium. It is the combined presence of these two membrane specializations which provide the epithelium with cable characteristics sufficient to conduct an action potential.

II. THE CONTROL OF CRUMPLING BEHAVIOR

a) Historical Perspective

The first thorough description of crumpling behavior was made by Romanes (1876) in observations of certain unspecified "discophorous species" of hydromedusae. He refered to this behavior as a "spasm" describing it as an "abnormally strong contraction of the swimming-muscles" and suggested that it serves to protect the animal or possibly to induce sinking as a result of the decreased surface area of the jellyfish. Romanes (1876) further observed that the "spasmotic" responses of these hydromedusae continue in the absence of the marginal nerve centers, and thus gave a first suggestion that epithelial conduction occurs in hydromedusae (although he assumed this was a direct stimulation of the effector muscle) and is involved with crumpling. It is interesting that in studies of *Sarsia* Romanes concluded that this jellyfish does not crumple but in common with scyphomedusae responds with an accelerated swimming if irritated. It is ironic that *Sarsia* was the animal Mackie and Passano (1968) used in demonstrating the involvement of epithelial conduction in the control of crumpling behavior.

In a subsequent study Romanes (1877) described crumpling in two leptomedusae *Tiaropsis* and *Staurophora*, as a "sudden and most violent contraction of the entire muscle sheet, the effect of which is to draw together all the gelatinous walls of the nectocalyx in a far more powerful manner than occurs during ordinary swimming...the whole bell thus assuming the form of an almost perfect square." In testing the sensitivity of different regions in eliciting a "spasm" it was noted that whereas only stimulation of the margin, tentacles, radial canals or manubrium of *Staurophora* produce the response, stimulation of any part of *Tiaropsis* invariably gives a strong spasm. Further studies of *Sarsia* showed that mechanical stimulation of the subumbrellar surface produces a retraction of the manubrium even in the absence of the margin. Furthermore, he found that whereas weak stimulation of a tentacle of *Sarsia* causes only that tentacle to retract, stronger stimulation of the same tentacle causes all tentacles and the manubrium to retract and still stronger stimulation causes the bell to respond with "one or more locomotor contractions". Since it is known that tentacular and manubrial retraction is associated with crumpling and that the radial muscle response of *Sarsia* is "inconspicuous except in the more responsive specimens" (Mackie and Passano, 1968), the above observations by Romanes (1877) may have been of a crumpling response. At the least there was preliminary evidence for the presence of excitable endoderm in *Sarsia*.

No subsequent description of this protective response appears to have been made until Hyman (1940) in behavioral observations of

several hydromedusae (the anthomedusae, *Stomotoca* and *Sarsia* and the leptomedusae, *Ballitaura* and *Phialidium*). It was noted that a "tap or blow" on the exumbrellar surface results in an escape response in which "the animal ceases pulsations, folds in the bell to the smallest possible compass and sinks". Differences in the sensitivity of the margin in activating crumpling between the medusae was also observed; *Ballitaura* was very sensitive, *Phialidium* and *Sarsia* moderately sensitive and *Stomotoca* was insesitive to anything but strong marginal stimulation. In *Sarsia* and *Stomotoca*, which have long manubria, stimulation of the manubrium was much more effective in eliciting crumpling than the margin.

Passano (1965) produced the first recordings of activity associated with crumpling in *Cladonema*. These recordings were from the margin probably representing epithelial potentials, although the source of excitation was not mentioned. No clear differentiation between the effectors for swimming and crumpling was made although an inhibition of swimming during crumpling, without affecting the marginal swimming pacemaker, is reported.

Mackie, Passano and Pavans de Ceccatty (1967) were the first to report the exact effectors of the crumpling behavior in *Sarsia*. This behavior was described as a simultaneous series of retractions of the tentacles, contraction of the marginal sphincter muscle and the four radial muscles and retraction of the manubrium. Using suction electrodes they showed that stimulation of the exumbrella produces impulses which are conducted throughout the exumbrella, subumbrella, tentacles, and manubrium during a crumpling response. It was

proposed that conduction in the subumbrella and tentacles was through the endoderm and that transfer between the exumbrellar ectoderm and endoderm was via transmesogleal bridges at the margin.

An extension of this study was made by Mackie and Passano (1968) in which electrophysiological evidence was provided for the excitability of exumbrellar ectoderm and endoderm and their involvement in initiating crumpling in *Sarsia*, *Euphysa* and *Phialidium*. It was clearly shown that the endodermal lamella conducts independently of the overlying swimming muscle and that excitation of exumbrella or subumbrella can conduct events to the opposite surface of the bell. Based on the observation of endodermal excitation associated with exumbrellar stimulated crumpling it was proposed that excitation of the radial muscle is through epithelial bridges between radial canal and muscle. Mackie and Passano (1968) also demonstrated that epithelial excitation normally elicits marginal activity in the form of pretentacle pulse (PTPs). In their description of crumpling the variability of the response was apparent. They indicated that exumbrellar excitation does not always produce a response and that sphincter muscle contraction occurs only during "full crumpling behavior" which is also associated with marginal (nervous) activity. Additionally, it was noted that there were often local responses of the manubrium and radial muscles without concurrent epithelial activity.

In an electrophysiological investigation of the anthomedusan *Spirocodon* Ohtsu and Yoshida (1973) showed that the exumbrellar ectoderm and the endodermal lamella are excitable but made no mention of the response associated with this conduction.

In a comparative analysis of the hydroid and medusa of the limnomedusan *Proboscidactyla* it was observed that stimulation of the colonial surface can induce crumpling of attached medusae (Spencer, 1974). The exact type of response obtained is dependent on the nature of the histological continuity between hydroid and medusa. Medusae with endodermal and ectodermal connections will either crumple or swim, medusae with only ectodermal connections always crumple, and attached medusae with only mesogleal connections show no response with colonial excitation. In unattached medusae stimulation of any part of the ectoderm results in the propagation of "crumpling pulses" (CPs) (Spencer, 1975). He noted that larger diameter stimulating electrodes and greater stimulus intensity is required to induce crumpling compared with marginal stimulation which readily elicits crumpling. In *Proboscidactyla* CPs conduct from the exumbrella to the subumbrella on a one to one basis and excite radial muscle in a stepwise manner. Since *Proboscidactyla* has endodermal radial muscles it may be difficult to apply some of these results to the control of crumpling in anthomedusae and leptomedusae which have ectodermal radial muscles.

The combined ultrastructural (Mackie and Singla, 1975) and electrophysiological (Mackie, 1975) study of the anthomedusan *Sarsia* has provided a good deal of information on the control of crumpling and neuro-epithelial interactions. The crumpling behavior in this jellyfish is substantially different from *Sarsia* in that it involves a simple and predictable stepped response of the radial muscles and retraction of the tentacles, manubrium and peduncular

wall. The crumpling effectors differ from those of other studied anthomedusae in that *Stomotoca* has no sphincter muscle and has smooth radial muscle fibers in the wall of the peduncle. Epithelial pulses (EPs) conduct on a one-to-one basis from the exumbrella to the subumbrella without apparent involvement of nerves and each EP of a series elicits a step in the radial muscle response. However, ultrastructural evidence is provided for the presence of synapses between nerves of the ectodermal peduncular plexus and the radial peduncular muscle and it is mentioned that frequent synapses occur between the radial nerves and radial muscle. The synapses between radial nerve and muscle could be related to the nervous control of the muscle during pointing, in which only one of the four muscles contract. As contraction of the peduncular muscle appears to occur only during crumpling, synapses onto the peduncular muscle suggest some involvement of nerves in the control of crumpling. Evidence is provided for the inhibition of nervous activity controlling the swimming and cryptic ring system by epithelial excitation.

A recent study of the excitation pathways mediating involution behavior of the siphonophore *Hippopodius* (Bassot *et al.*, 1978) should be considered due to the similarity of the response to crumpling. Involution of the nectophores (medusoid individuals) involves an inward curling of the margin due to contraction of the radial ectodermal muscle of the velum and the radial endodermal muscle of the psuedovelum (the proximal region of the subumbrella). Both the exumbrellar ectoderm and subumbrellar endoderm are excitable and stimulation of either of these regions produces involution.

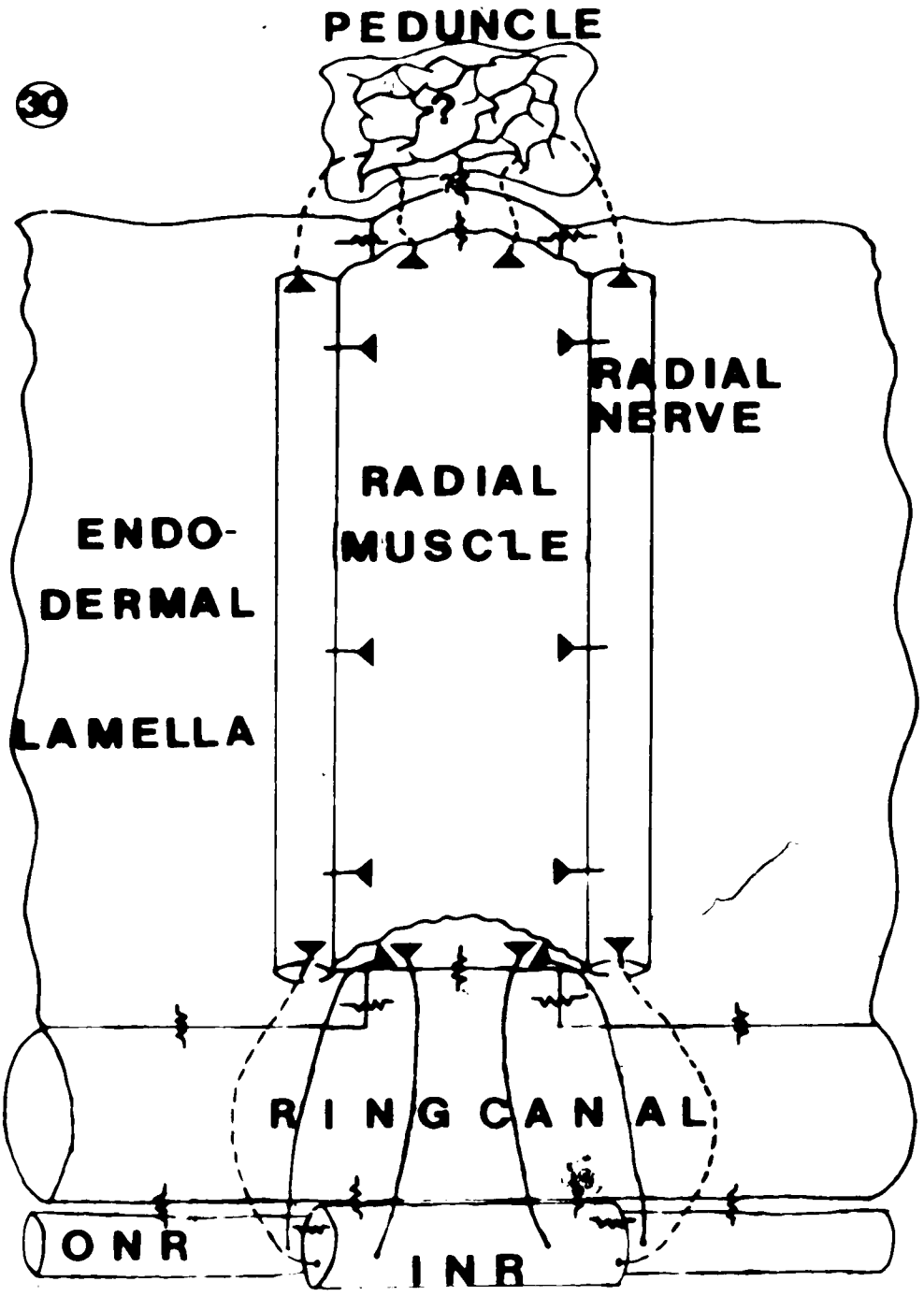
By cutting through the exumbrellar ectoderm entirely around the margin, it was shown that stimulation of this isolated ectoderm or of intact ectoderm outside of the incision will elicit involution. This suggests that there are two sites of functional ectodermal-endodermal connections; one at the margin, as proposed in hydromedusae, and another at the apex of the nectophore where the axial canal enters the subumbrellar cavity from the stem. Conduction between ectoderm and endoderm is polarized at the margin, in the exumbrellar to subumbrellar direction, but not at the apical end of the bell, where conduction in either direction can occur. It is noted (Bassot *et al.*, 1978) that although stimulation of the epithelial surface of a single nectophore will elicit involution possibly with an entirely epithelial pathway, conduction of the response to nectophores requires nervous intervention. Apparently, epithelial pulses of a nectophore excite nervous activity in the stem which in turn is retranslated into epithelial excitation at other nectophores. These epithelial-neural and neural-epithelial pathways may require facilitation.

b) Anatomical Interpretation with Ultrastructural Findings

Figure 30 outlines the probable anatomical connections which incorporate the physiological and ultrastructural results of this study. Note that connections denoted by dashed lines are those which are consistent with the physiology but of which no firm structural data exists.

One possible way of correlating the physiological pathways exciting radial muscle with known structural connections is to consider that there is a direct excitation of radial muscle by

FIGURE 30 Wiring diagram of radial muscle excitation pathways. Diagram of possible histological connections which support the physiological evidence for the presence of marginal and apical radial muscle excitation pathways. Not drawn to scale. Symbols: ? - presence of ectodermal nerve plexus in the peduncle and epithelial connections between endoderm and peduncular ectoderm are unproven; resistors - electrotonic connections; triangles - chemical synapses; solid lines - established connections; dashed lines - unestablished connections.


30
PEDUNCLE
RADIAL NERVE
RADIAL MUSCLE
ENDO-DERMAL LAMELLA
RING CANAL
ONR
INR

electrotonic spread from the endoderm. This would incorporate the previous proposals for activation of radial muscle (Mackie *et al.*, 1967; Mackie and Passano, 1968; Mackie, 1975; Mackie and Singla, 1975). However, to make this hypothesis fit the established marginal and apical initiation sites of radial muscle potentials (RMPs) would require that only those connections between radial muscle and radial canal at the marginal and apical ends of the muscle are functional. This then demands that despite the fact that the epithelial unions between radial muscle and canal appear structurally identical throughout the perradius only the marginal and apical bridges have characteristics which allow sufficient current flow from endoderm to excite radial muscle. If indeed this hypothesis were tenable it would be a novel circumstance in that there is an excitation of muscle (albeit myoepithelial) by an epithelium, counter to the established dogma of nerves exciting muscle.

The more consistent hypothesis is that no direct endodermal excitation of radial muscle occurs, but that activity of the muscle is initiated through synaptic output of nerves. There are two possible ways in which nervous activity could exert a control over radial muscle response during crumpling. Firstly, there may be an excitation of the muscle through transmission from radial nerves, which synapse onto radial myoepithelial cells throughout the perradius. To explain the marginal and apical RMP pathways there must be some way of exciting the radial nerves at these two regions of the perradius. Although the anatomical relationship between the radial nerves and marginal nerves has not been established, the

observation of physical connection and transport of fluorescent dye between inner nerve ring (INR) and radial nerves in *Polygordius* (Spencer and Satterlie, personal communication) is ample evidence for assuming the continuity of radial and marginal nerves. Thus with epithelial excitation the INR neurons could excite radial muscle through the marginal pathway. For the apical RMP pathway the best suggestion is that there is a connection between radial nerves and an ectodermal nerve plexus in the peduncle. Such a plexus exists in *Stomotoca* (Mackie and Singla, 1975) but has not been searched for in *Polygordius*.

An alternative means of nervous control over radial muscle activity during crumpling could occur by direct neuronal transmission to the muscle at its marginal and apical ends. Excitation would then have to pass along the muscle through myoid electrotonic spread between myoepithelial cells. As evidence for this, synapses occur between INR neurites and radial muscle processes at the margin. Additionally because there appears to be a union between radial muscle and sphincter muscle at a margin, synapses between outer nerve ring (ONR) neurites and smooth muscle could also represent a means of exciting radial muscle. This alternative would also require that there be some innervation of apical radial muscle, perhaps by nerves from a peduncular nerve plexus although no evidence for this exists. One inadequency of this explanation is that gap-junctions have not been observed between radial myoepithelial cells which would probably be required for intercellular current flow between cells. However, freeze-fracture and lanthanum impregnation of radial muscle is needed to confirm whether or not there are gap-junctions.

Regardless of the exact means of radial muscle activation by nerves with subumbrellar stimulation there must be an excitation of nerves by endoderm during crumpling. For the marginal pathway this probably involves an electrotonic spread from ring canal to marginal nerves. Epithelial connections between ring canal and ectoderm occur at both the subumbrellar and exumbrellar sides of the marg[redacted] This would represent a means of exciting either INR or ONR neurons. For the apical pathway there must be similar electrotonic bridges between endoderm (probably radial canal) and peduncular ectoderm.

The two possible types of nervous excitation of radial muscle activity are not necessarily mutually exclusive. It is possible that marginal and apical nervous transmission occurs in conjunction with transmission from radial nerves. Alternatively, the marginal and apical pathways may involve different excitation routes as suggested by the quicker fatigue of the apical pathway. It is conceivable, because of its greater lability, that apical excitation involves an activation of radial muscle through radial nerves (endoderm → nerve → nerve → muscle) whereas the marginal pathway proceeds through the more direct excitation of muscle via marginal nerves (endoderm → nerve → muscle).

The above discussion has entailed the crumpling pathways active during subumbrellar stimulation. Exumbrellar stimulation produces a more variable crumpling response which can involve any of the following patterns:

1. A full crumpling behavior involving all of the crumpling effectors; radial muscle, sphincter muscle, longitudinal muscle of the tentacle and manubrium, and radial velar muscle.

2. A partial crumpling behavior, notably without sphincter muscle contraction, involving contraction of the radial muscles and longitudinal muscles of the tentacle, and sometimes twitching of the manubrium and velum.
3. A initial partial crumple (perhaps requiring several stimuli) leading to a full crumple after several shocks.

The lability and variability of the crumpling response is suggestive of an intermediate nervous pathway between ectoderm and radial muscle and incongruous with the through-conducted epithelial pathway proposed in other hydromedusae (Mackie and Passano, 1968; Mackie, 1975; Spencer, 1975). In addition, the frequent requirement for the facilitation of several exumbrellar pulses (EPs) in order to excite radial muscle would seem to demand a nervous route, although the epithelial excitation of luminescence in *Hippopodius* appears to involve facilitation (Bassot *et al.*, 1978). The strongest evidence for the existence of nervous excitation of radial muscle in *Polyorchis* is the activation of the muscle in the absence of endodermal excitation. The only other explanation of this might be a direct ectodermal activation of radial muscle at the margin and myoid spread along the muscle. Even when endoderm is excited by EPs, as during a full crumpling response, this is usually subsequent to radial muscle twitches.

The presence of two distinct patterns of the crumpling response initiated by exumbrellar stimulation suggests different consequences of EPs conducted to the margin. When radial muscle is excited in the absence of or prior to endodermal activity, an EP may excite the muscle directly or more likely excites nerves which

synapse with radial muscle. When endoderm is excited by exumbrellar stimulation there must be an electrotonic spread across the mesoglea from either exumbrellar or subumbrellar ectoderm. The association of endodermal and sphincter muscle activity implies that they are part of a common excitation pathway. It can be speculated that when exumbrellar stimulation elicits full crumpling EPs excite sphincter muscle, either through nerves or directly, and that this is the source of electrotonic spread to the ring canal through epithelial bridges.

One inconsistency with the proposal of nervous activation of radial muslce is the preliminary finding that excess Mg^{++} (1:1, $MgCl_2$ and seawater) doesn't block crumpling. Although after 1-2h in $MgCl_2$ the observed contractions of radial muscle are feeble and the RMPs greatly reduced in amplitude, both marginal and apical pathways can be demonstrated. It is well documented that excess Mg^{++} inhibits nervous excitation of swimming muscle and longitudinal muscle of the tentacle (Mackie and Passano, 1968; Ohtsu and Yoshuda, 1973; Mackie, 1975; Spencer, 1975, 1978). Although this suggests an epithelial excitation of radial muscle it is not possible to determine the exact site(s) of action of Mg^{++}. Proof of nervous or epithelial activation of radial muscle will have to wait for the use of inhibitors more specific to neuromuscular junctions or intracellular recordings.

More evidence suggesting the involvement of nerves during the crumpling of *Polyorchis* is the observed excitation of PTPs. This phenomenon was also observed in *Aurelia* where it was noted that the

full crumpling response always involves marginal excitation (Mackie and Passano, 1968). It is suggested by Mackie and Passano (1968) that this nervous activation may be important in either further exciting the epithelium, thus reinforcing the crumpling response, or in directly exciting the crumpling effectors. Although multiple epithelial excitations were never observed in *Polyorchis* it is obvious that tentacle retraction (associated with PTPs) is a part of the crumpling response and may signify the excitation of a common nervous pathway which also activates radial muscle contraction.

To explain the three types of innervation of radial muscle, it must be remembered that crumpling is not the only response which employs radial muscle contraction. The feeding or pointing behavior in hydromedusae involves the contraction of a single radial muscle and simultaneous retraction of the manubrium (Romanes, 1877; Mackie, 1975; Mackie and Singla, 1975). This unilateral or asymmetrical control of radial muscle response must require nerves which would likely be separate from the nerves controlling crumpling.

In order to hypothesize the nervous control of crumpling it is necessary to explain how epithelial activity can produce nervous excitation. Indeed, there is precedence for active interactions between epithelia and nerves. Skin impulses of frog larvae must excite sensory neurons to activate swimming (Roberts and Stirling, 1971; Roberts, 1971). The activation of trunk myotomes by skin pulses in larvaceans (Bone and Mackie, 1975) requires the passage of depolarizing current from epithelia to sensory nerves. The inhibition of the swimming myotomes by skin impulses in ascidian tadpoles

(Mackie and Bone, 1976) suggests the passage of hyperpolarizing current between epithelia and motor or central neurons. Although stimulation of the surface of a nectophore of *Hippopodius* may elicit an involution of that individual through an epithelial pathway, conduction of the response requires an epithelial excitation of nervous activity in the stem and re-excitation of epithelial pulses in other nectophores (Bassot *et al.*, 1978). Synaptic transmission from motoneurons results in the production of excitatory postsynaptic potentials (EPSPs) and spikes in gastropod salivary gland cells, serving to coordinate salivation with feeding behavior (Kater *et al.*, 1978a). Finally, activity of segmental nerves can excite the photocytes of a polynoid polychaete (Herrera, 1979).

If in fact the hypothesis of Mackie and Passano (1968) that radial muscle activity results from an electrotonic spread from endoderm is correct, this would be a novel circumstance in which epithelial excitation directly produces an active, muscular response. Although there is physiological evidence that involution in *Hippopodius* is mediated epithelially, no histological evidence exists to support the presence of epithelial connections required for this hypothesis (Bassot *et al.*, 1978). Additionally, there is no indication that nervous intervention exists in eliciting endodermal secretion or ectodermal bioluminescence in *Hippopodius* (Mackie, 1976b; Bassot *et al.*, 1978). However, this is a different type of response, in which the epithelium serves both as the conducting substrate and the effector. It may be that the release of a secretory product or emmission of luminescence is simply a metabolic byproduct of excitability and that the responses have been conserved through

evolution because of the adaptive advantages that accrue.

Despite the many examples of epithelial initiation of nervous activity, no theories have been constructed to explain the cellular basis of this interaction. The marginal neurites of hydromedusae, especially those of the CNR, are frequently ensheathed by ectodermal processes. It is conceivable that epithelial excitation may produce a depolarization of the neurite by alteration of the surrounding ionic medium. Baylor and Nicholls (1969) have reported a depolarization of tightly encompassing glial cells due to K^+ buildup following nervous activity. Mackie (1975) has suggested an analogous mechanism for producing an opposite effect; a hyperpolarization of swimming pacemaker neurons during epithelial excitation in *Aglantha*. There is also an inhibition of swimming by epithelial excitation in *Polyorchis*. Intracellular recordings from INF neurites have shown hyperpolarizing inhibitory postsynaptic potentials (IPSPs) when the epithelium is stimulated (Spencer, personal communication). This is direct evidence for an alteration in neuronal membrane potential by epithelial excitation.

The initiation of nervous activity by epithelial excitation may be analogous to the epithelial-neural interactions that occur during sensory transduction. Vertebrate auditory, gustatory and vestibular sensory systems have specialized peripheral epithelial (Merkel) cells closely associated with sensory nerve fibers (reviewed by Munger, 1977). Although there is little physiological evidence for the types of interactions that occur it is apparent that there must be some form of excitation transfer from the Merkel cell to the nerve in order to transmit a sensory stimulus.

c) Function[redacted] of the Crumpling Pathways to the Intact Jellyfish

In the first place, it must be emphasized that the verification of marginal and apical crumpling pathways, possibly neuronal, in *Polyorchis* does not signify that they exist in all or even most hydromedusae. It is conceivable that *Polyorchis* is peculiar among hydromedusae in this regard. Nonetheless, I feel that the presently accepted epithelial excitation of radial muscle in the other medusae that have been studied (Mackie and Passano, 1968; Ohtsu and Yoshida, 1973; Spencer, 1975; Mackie, 1975) should be reappraised in light of the results of this study. Particularly, I must stress the importance of recording directly from radial muscle, which heretofore has not been done, in order to establish the excitation pathways.

As it has generally been assumed that the endodermal lamella functions as a conducting layer for eliciting an exumbrellar initiated crumpling response, a new function for this tissue layer must be suggested. It seems likely that the lamella, at least in *Polyorchis*, acts as a receptive field and conduction pathway for subumbrellar initiated crumpling. Since the subumbrellar ectoderm serves as the swimming muscle, this outer layer can not perform a sensory or conductile function in mediating crumpling. The histological location of the endodermal lamella, situated beneath a layer of ectoderm and mesoglea, will reduce the sensitivity of the subumbrellar surface to mechanical stimulation. However, due to the tall shape of the bell of *Polyorchis* it is not very likely that an intruding organism would disturb the general subumbrellar surface. Such stimulation would be more common in leptomedusae due to their flat saucer-shaped bell.

In *Polyorchis* and other anthomedusae, it is more likely that there would be agitation of the manubrium in nature due to the presence of a long, pendant manubrium which, when relaxed, extends well below the bell margin. This also suggests a possible significance for the presence of the apical radial nerve pathway. Severe irritation of the manubrial tip would be expected to excite endodermal pulses which, upon conduction to the peduncle, would initiate crumpling. The latency of this response would be considerably greater if the endodermal excitation had to pass to the margin. It may be relevant that Hyman (1940) noticed that crumpling was most readily activated by manubrial stimulation in the anthomedusae *Tubularia* and *Corymorpha*. Additionally, the apical pathway may be important in initiating crumpling by a stimulation of the gonads which in *Polyorchis* are very long and also extend below the bell margin in mature individuals.

Gentle stimulation of the general exumbrellar surface of *Polyorchis* would not be expected to produce a crumpling response. It is expected that severe and repeated agitation of the exumbrella would be needed to activate a crumple in nature. The requirement for facilitation of EPs in activating crumpling may be significant to the animal. Because medusae are generally at the mercy of tidal currents, there will be a frequent contact of the exumbrellar surface with non-aggressive or inanimate objects severe enough, perhaps, to excite the ectoderm. An aggressive attack on a jellyfish by another animal, on the other hand, will likely involve numerous, acute agitations of the exumbrella. It can be speculated, therefore,

that the demand of crumpling for facilitation of EPs may be adaptive in conserving muscular output for circumstances that present a real potential harm for the jellyfish. In addition, needless crumpling would impair the performance of other important behaviors, such as swimming and feeding. On the other hand, marginal irritation, which might represent the greatest potential harm, should easily elicit a full crumpling response.

In conclusion, it is suggested that although *Polyorchis* is distinguished from most higher animals in possessing excitable epithelia which serve as sensory fields for the activation of a protective crumpling response, this response is initiated by neuronal mechanisms similar to those of higher animals.

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PLATE 1 General morphology of the perradius.

a) Light micrograph of transversely sectioned perradius showing the endodermal radial canal (rc), at a lateral extension (le), attached at both ends with the endodermal lamella (el) separated by a thin layer of mesoglea (m) from the ectodermal radial muscle (rm), with lateral radial nerves (rn), and the swimming muscle layer (sm). X1000.

b) Transverse section through a radial nerve bundle (rn) apposed to radial muscle (rm) and swimming muscle (sm). X4000.

c) Peripheral radial canal nerves (cn) seen in transverse section near the mesoglea (m). These nerves are often ensheathed by the endodermal cells containing a scattered array of basal myofibrils (mf). This section is from lanthanum-impregnated tissue and 3 gap-junctions (arrowheads) can be seen. X24,000.

d) Radial canal at the mesoglea (m) showing the circular smooth myofilaments (mf) and several gap-junctions (arrowheads) between the basal ends of the endodermal cells. X45,000.

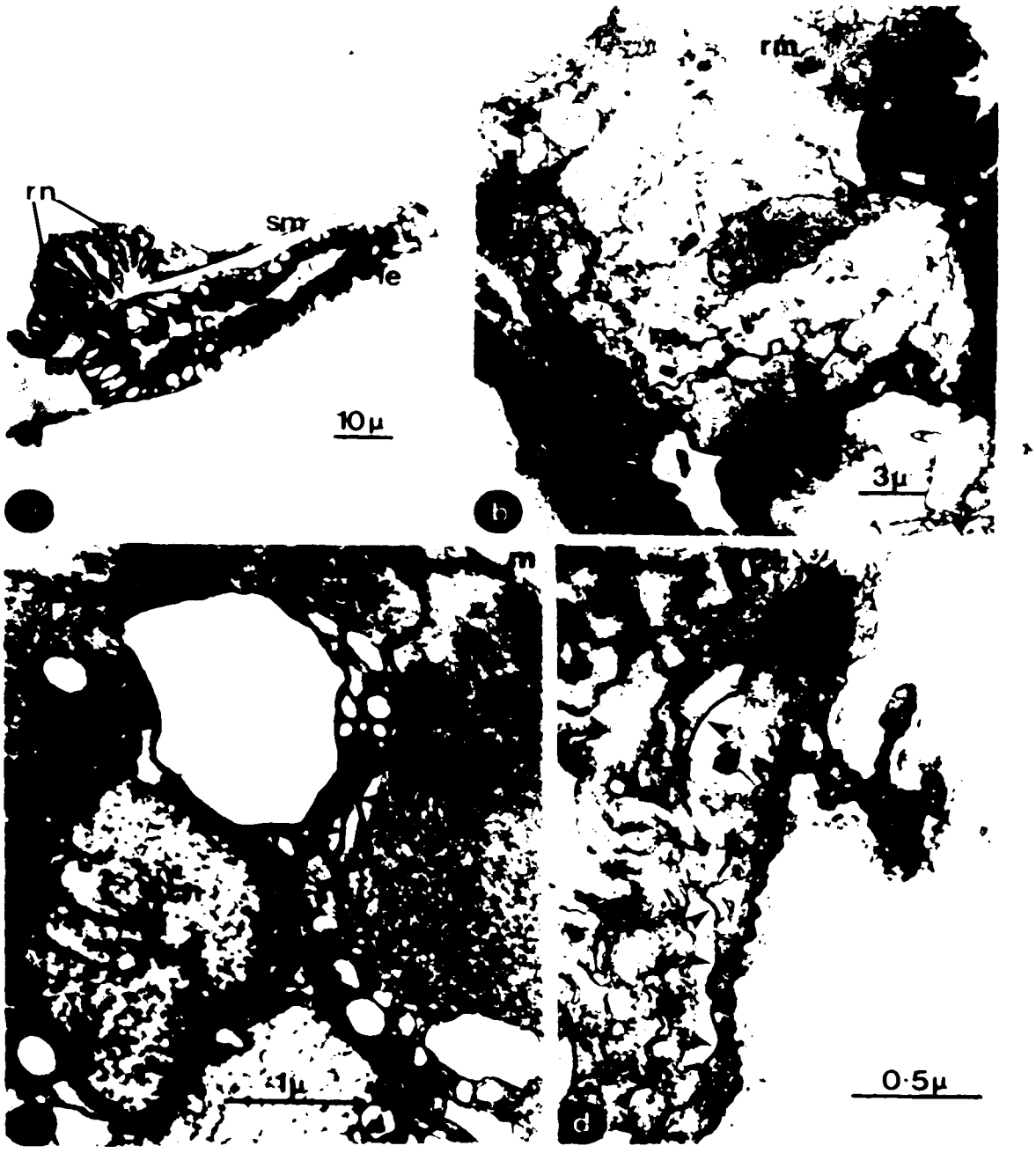
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PLATE 2 General morphology of the interradial margin.

a) Light micrograph of a radial section through the margin showing the triradius where exumbrellar (below), subumbrellar (above) and velar (at the left) mesoglea converge. Symbols: ee - exumbrellar ectoderm, el - endodermal lamella, inr - inner nerve-ring, onr - outer nerve-ring, rnc - ring canal, sm - swimming muscle, spm - sphincter muscle, t - triradius, te - ectoderm of a tentacle, v - velum. X1000.

b) Radially sectioned ectodermal sphincter muscle (spm.) and overlying neurites from the outer nerve-ring (onr) separated by a thin layer of mesoglea (m) from the endodermal ring canal (rnc) with peripheral canal nerves (cn). X5000.

c) Base of the velum cut in radial section showing the striated, circular swimming muscle (sm) on the subumbrellar side, meseglea and smooth, poorly developed radial myofibrils (mf) on the exumbrellar side. X9000.

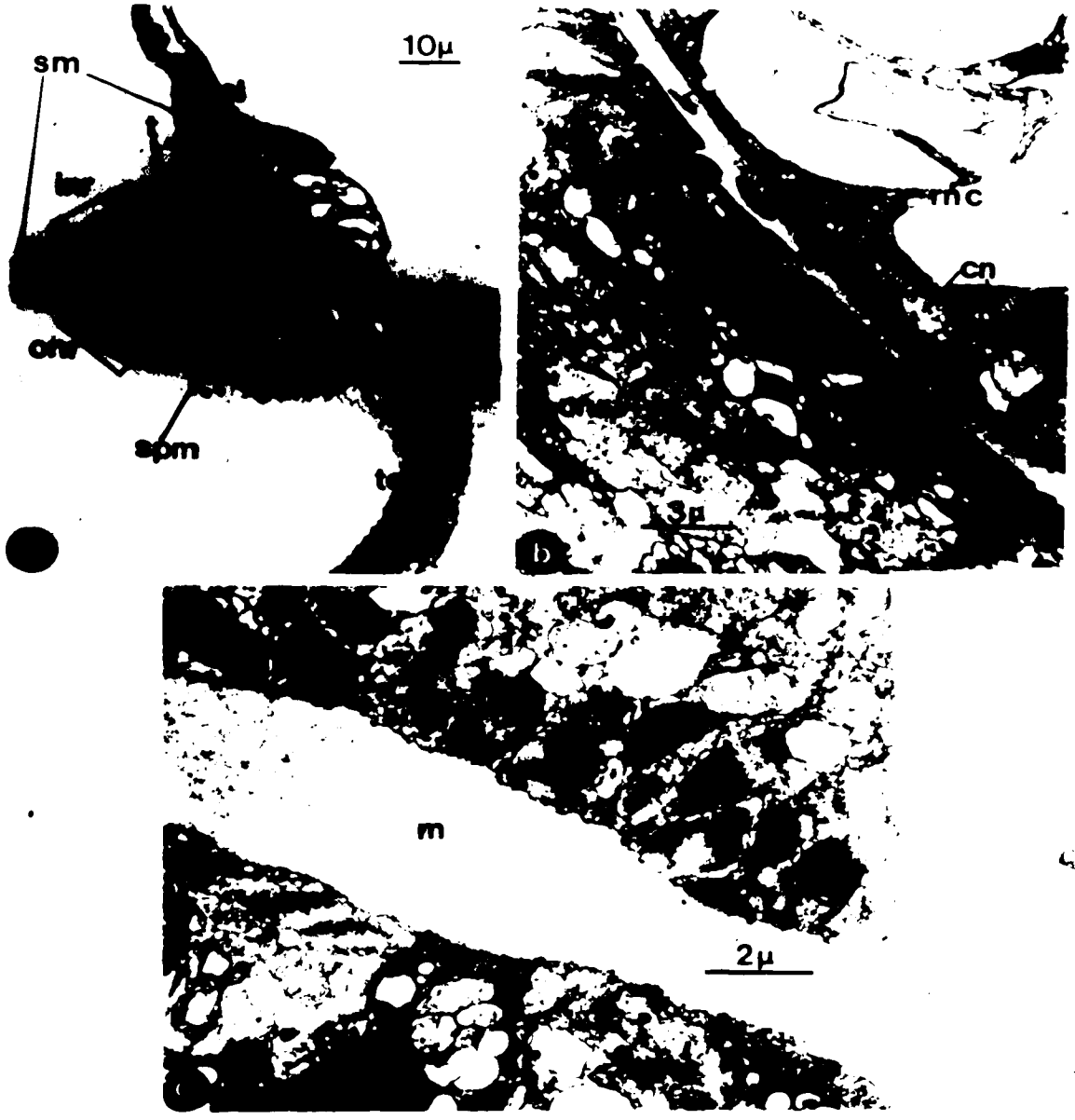
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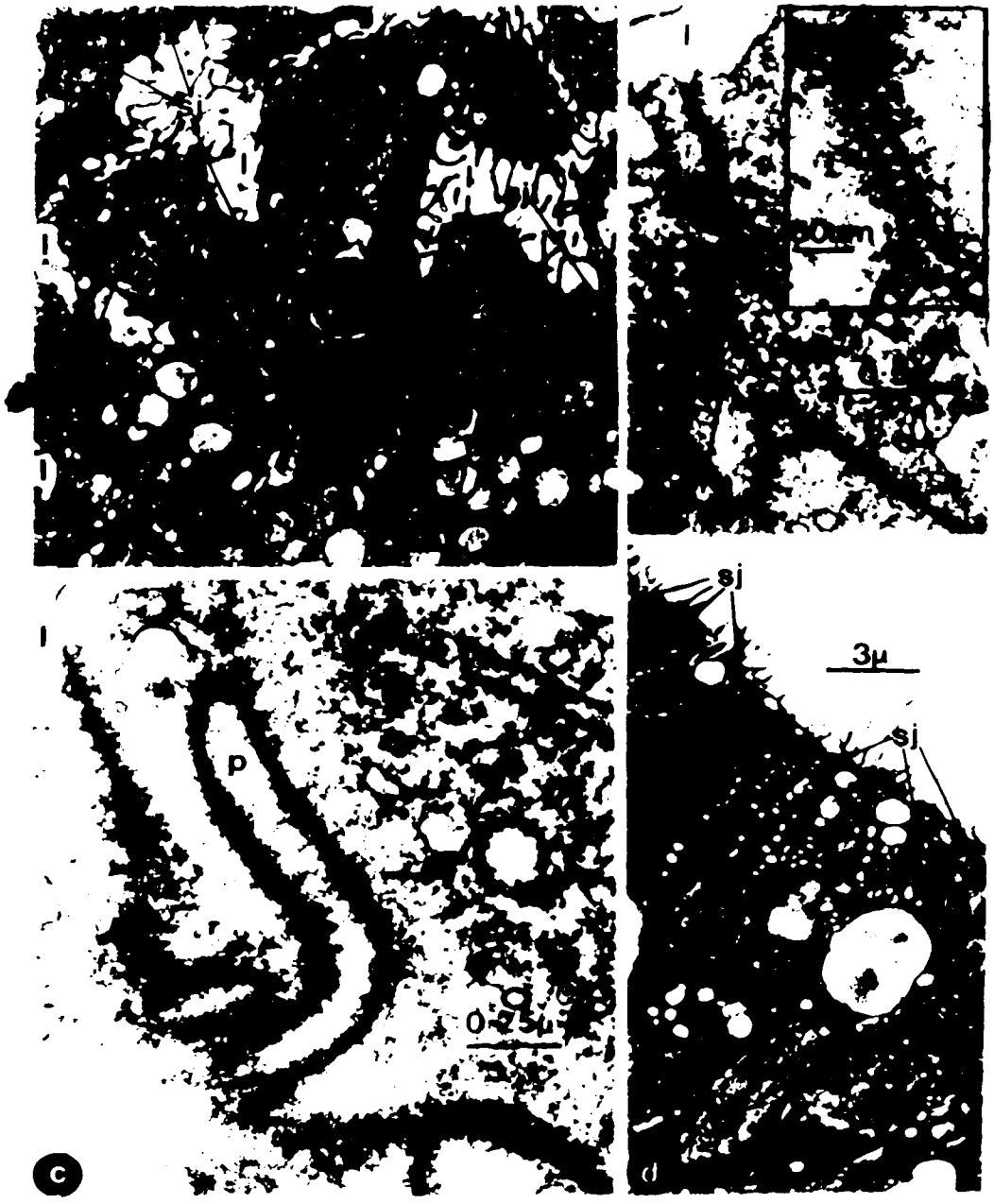
PLATE 3 Localization and structure of septate junctions in the endodermal canal.

a) Lanthanum-impregnated and uranyl acetate/lead citrate strained endodermal canal. Septate junctions (sj) between endodermal cells originate at the lumen (l) and extend short distances (shown by the bracket). X7000.

b) Lanthanum-impregnated and uranyl acetate/lead citrate stained endodermal canal. Septate junction at the lumen (l) enclosing interdigitating processes (p). Note the relative absence of extracellular lanthanum. X46,000. Inset X190,000.

c) Lanthanum-impregnated and uranyl acetate/lead citrate stained endodermal canal. Septate junction at the lumen (l) in tangential section showing the non-pleated, concentric arrangement of septa (arrowheads) and a longitudinally sectioned interdigitating process (p). X62,000.

d) Conventionally fixed and stained margin at the exumbrellar surface. Septate junctions (sj) enclosing cellular processes occur between the apical ends of the ectodermal cells. Note the presumably sensory cilia and processes associated with neurites (n) from the outer nerve-ring. X5000.


sj
sj
3μ
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0·25μ
c
d

PLATE 4 Localization of and conventionally stained gap-junctions in the endodermal canal.

a) Lanthanum-impregnated and uranyl acetate/lead citrate stained endodermal canal at its periphery showing an array of gap-junctions (arrowheads) between the basal ends of endodermal cells. Mesoglea (m) surrounds the canal and its connection (c) with the endodermal lamella. X20,000.

b) Conventionally stained and fixed endodermal canal. Two gap-junctions (gj) in transverse section with an intercellular space of about 4.5nm. X111,000. Inset X240,000.


m
c
50 nm
2 nm

PLATE 5 Lanthanum-impregnated endodermal gap-junctions in transverse section.

a) Series of gap-junctions (arrowheads) within an endodermal process midway in endodermal canal-endodermal lamella connection. Note that at regions where the junctions are twisted lucent globules (g) are apparent. X20,000.

b) Higher magnification of a region of a) showing the striated appearance of transversely sectioned, lanthanum-filled gap-junctions. X85,000. Inset shows electron-lucent bridges (b) between plasma membranes. Within a bridge a electron-dense line (arrowed) is seen traversing the junction. X410,000.

PLATE 6 *En face* arrays of lanthanum impregnated endodermal gap junctions.

a) *En face* section (not grid stained) of a gap junction within the endodermal canal. The electron lucent globules are not well organized in that there are numerous irregular spaces within the junction. X110,000.

b) *En face* section (not grid stained) showing 3 gap junction plaques within the endodermal canal. In areas where they are more tightly packed, the globules often appear to be hexagonally arranged. X140,000.

c) Uranyl acetate/lead citrate stained gap junction within the endodermal canal seen in *en face* section. This junction is loosely packed as seen by the numerous electron-dense areas between globules. The arrows indicate globules in which a star-like shape can be recognized, suggesting they are composed of subunits. An electron-dense dot at the center of the globules can be seen. X330,000.

d) Uranyl acetate/lead citrate stained endodermal lamella showing a tight association between gap-junctions (arrows) and septate junctions (sj). X55,000.

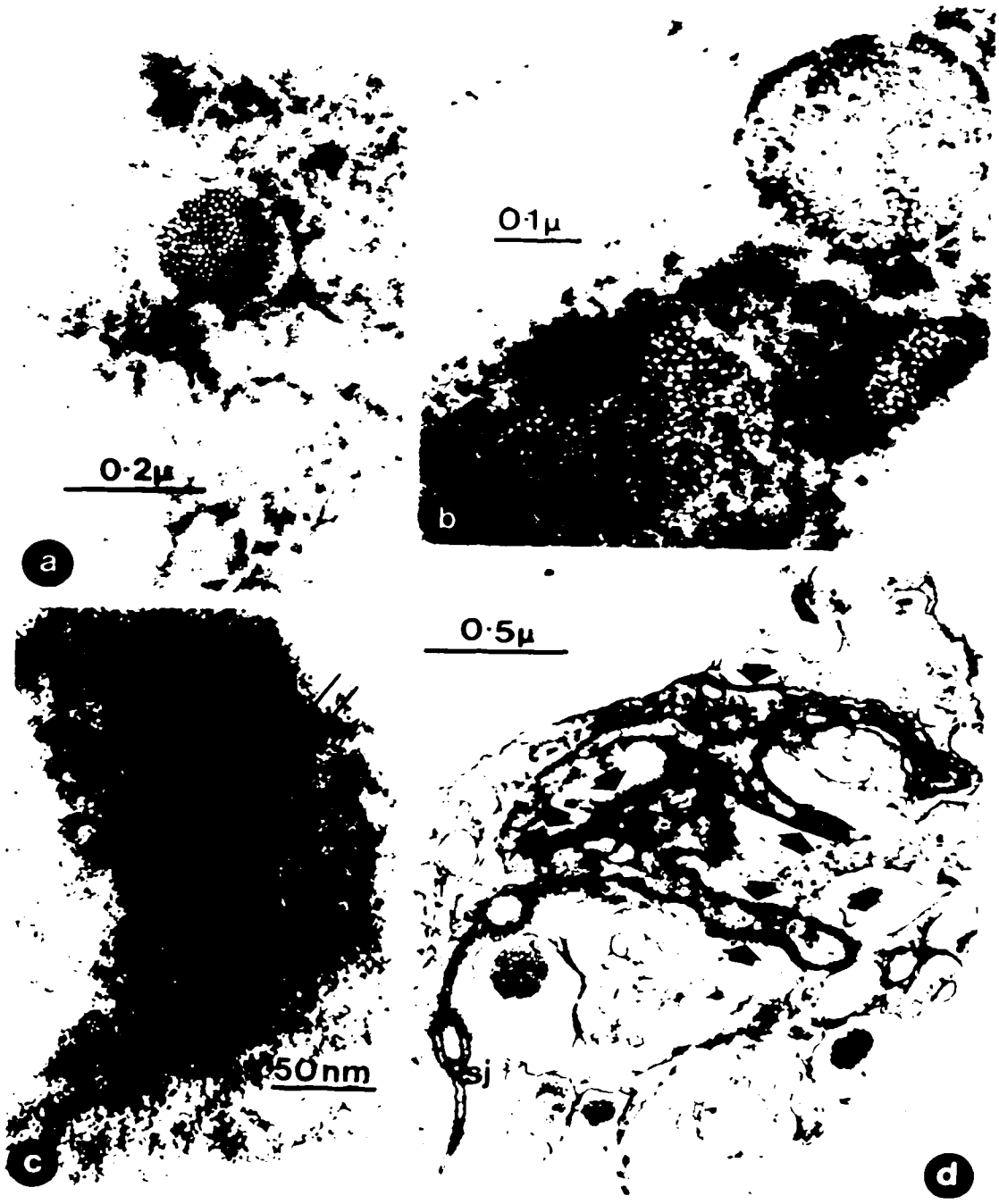

0·2μ
a
0·1μ
b
0·5μ
50nm
c
sj
d

PLATE 7 Freeze-fracture replication of septate junctions.

a) Series of septate junctions (sj) in a replica from the radius. These junctions have rows of EF particles (about 10nm in diameter). As in sectioned material these septate junctions often enclose interdigitating processes (p) [redacted] most common at the surface of the tissue, indicated here by their proximity to surface extensions (e). X38,000.

b) c) Higher magnifications of the septate junction in a) indicated by the arrow. In b) a correspondence between the EF particle rows (showing a periodicity of about 12nm) and PF grooves (arrowhead) can be seen. X85,000.

Symbols: ef - exoplasmic face, pf - protoplasmic face, Circled arrow at lower left shows the shadow angle.

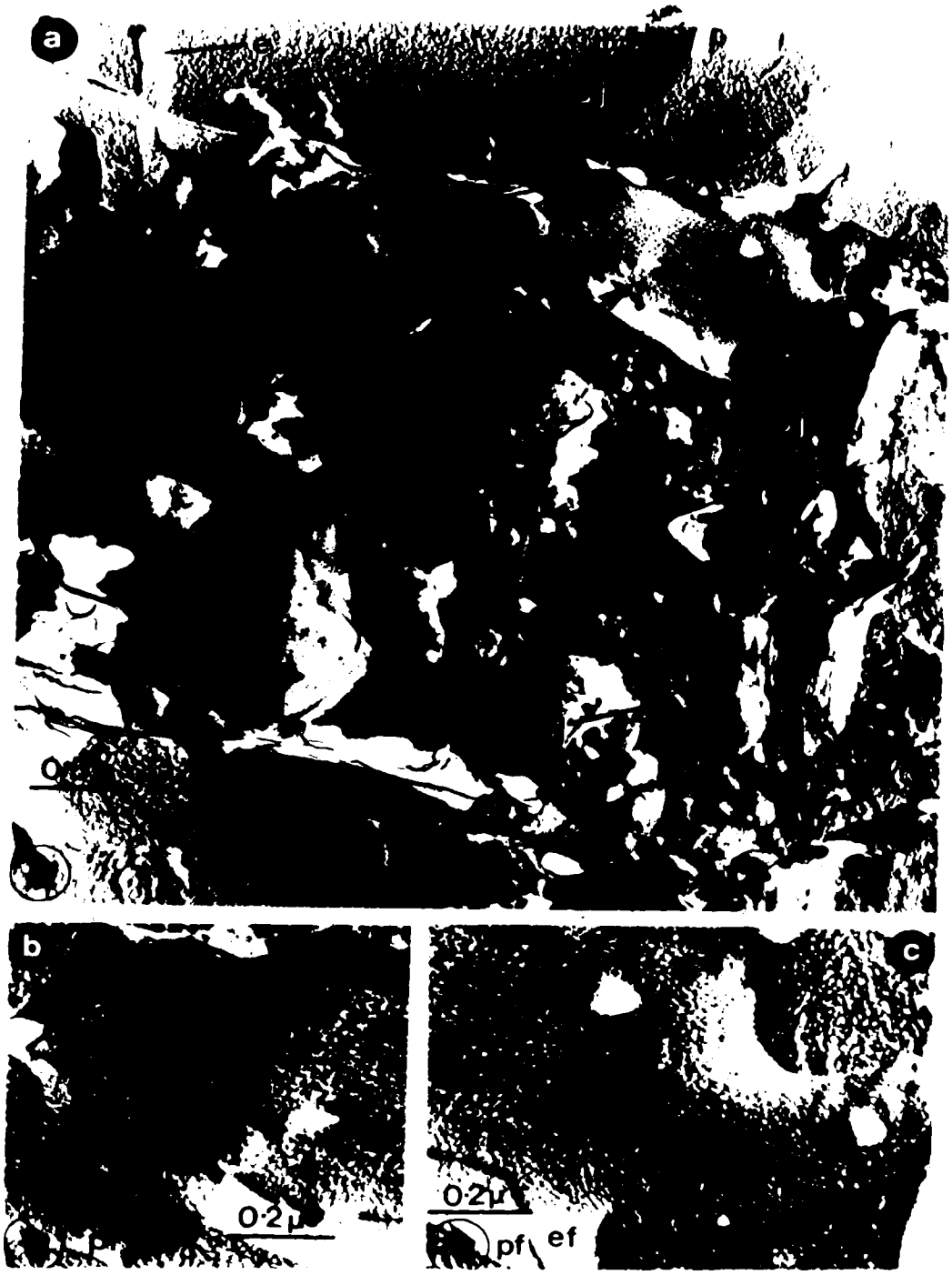

a
b
0·2μ
c
0·2μ
pf
ef

PLATE 8 Freeze-fracture replication of gap-junctions with irregular particle aggregates.

a) Replica of marginal tissue showing several EF particle aggregates (arrowheads) representing gap-junctions. These aggregates have irregular arrangements of particles. Although many of the arrays have regions in which the fracture plane deviated to the PF, no complementary PF pits have been seen. X20,000.

b) Enlargement of the gap-junctions labelled "b" in a). X79,000.

c) Enlargement of the gap-junctions labelled "c" in a). X79,000.

Symbols: ef - exoplasmic fracture face, pf - protoplasmic fracture face, circled arrow at lower left shows the shadow angle.


a
0·5μ
c
er
b
c

PLATE 9 Freeze-fracture replication of gap-junctions with regular (plaque-like) arrangement of particles.

a) Replica of marginal tissue showing several plaque-like EF particle aggregates presumed to be gap-junctions. These aggregates display a tight packing of particles comparable to the *en face* sectioned lanthanum-filled plaques of Plate 6b. No complementary pits are seen on the PF within some of the plaques. X33,000.

Inset 1 is an enlargement of the particle aggregate labelled 1 in a). X150,000. Inset 2 is an enlargement of the particle aggregate labelled 2 in a). X166,000.

b) Replica of marginal tissue showing an EF particle aggregate. The particle diameters and periodicity and the presence of particle-free patches within the aggregate show a close correspondence to the *en face* arrays of lanthanum-impregnated gap-junctions in Plate 6. X120,000.

c) Enlargement of the gap-junction of b). Several particles suggest a star-like shape (boxes) and the presence of a central depression (arrowheads) similar to but not as distinct as the negative stained globules of Plate 6c. X210,000.

Symbols: ef - exoplasmic fracture face, pf - protoplasmic face, circled arrow at lower left shows the shadow angle.

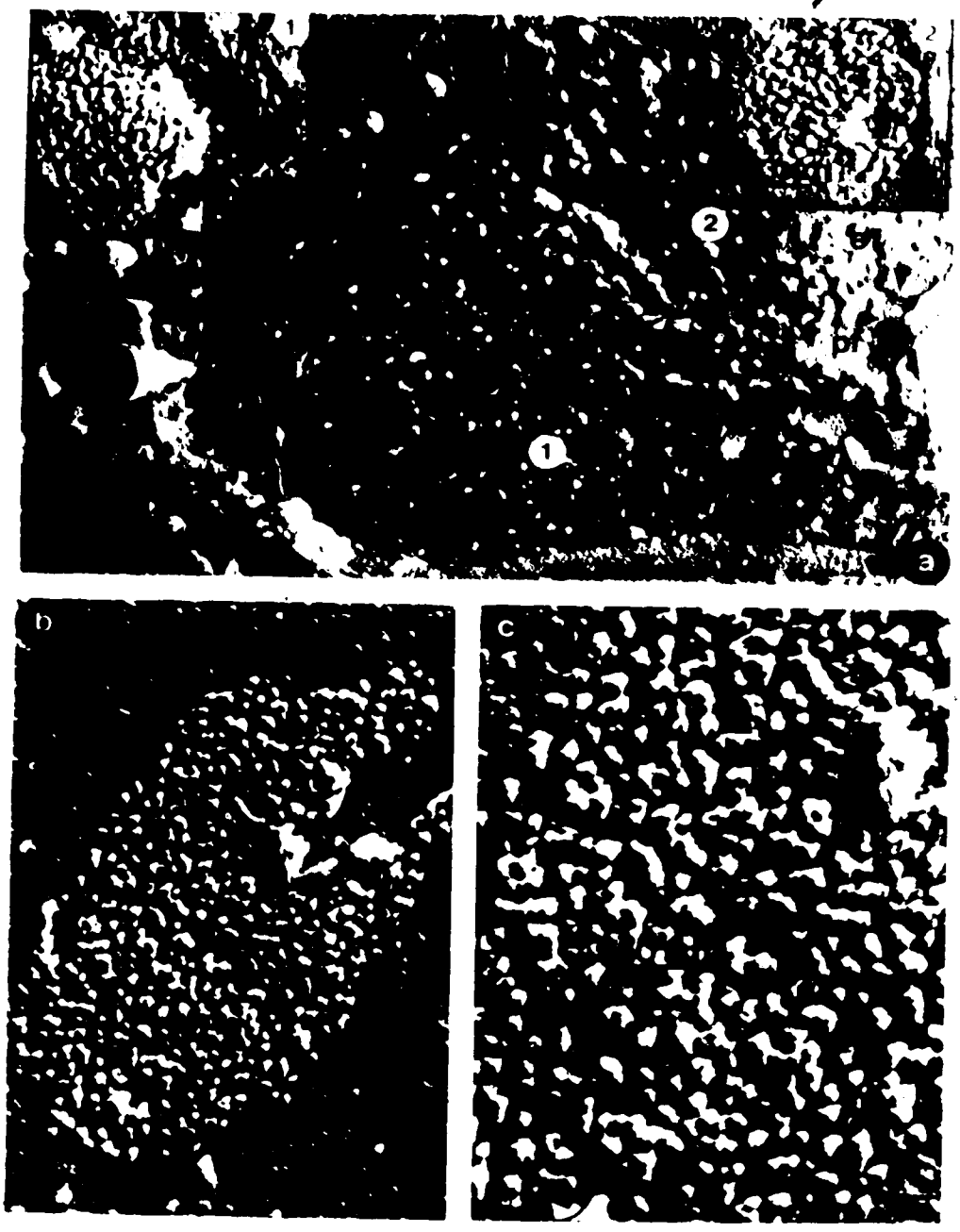

1
2
a
b
c

PLATE 10 General ultrastructure of radial muscle.

a) Transverse section of radial muscle showing the invagination of mesoglea (m) into the muscle almost as far as the subumbrellar surface (su). X7000.

b) Higher magnification of transversely sectioned radial muscle in which "desmosome-like" junctions (arrowheads) between the basal ends of the myoepithelial cells are seen. Note that the myofibrils of some cells are arranged in distinct aggregates (1) whereas those of other cells are more loosely packed but homogeneously arranged throughout the basal region of the cell (2). X13,000.

c) Enlargement of the "desmosome-like" junctions present between radial myoepithelial cells. At regions (arrowheads) electron-dense filaments can be seen traversing the intercellular space (ca. 10nm) between apposed cells. It is assumed that these junctions serve a mechanical function. Small aggregates of microtubules (boxes) can also be seen. X85,000.

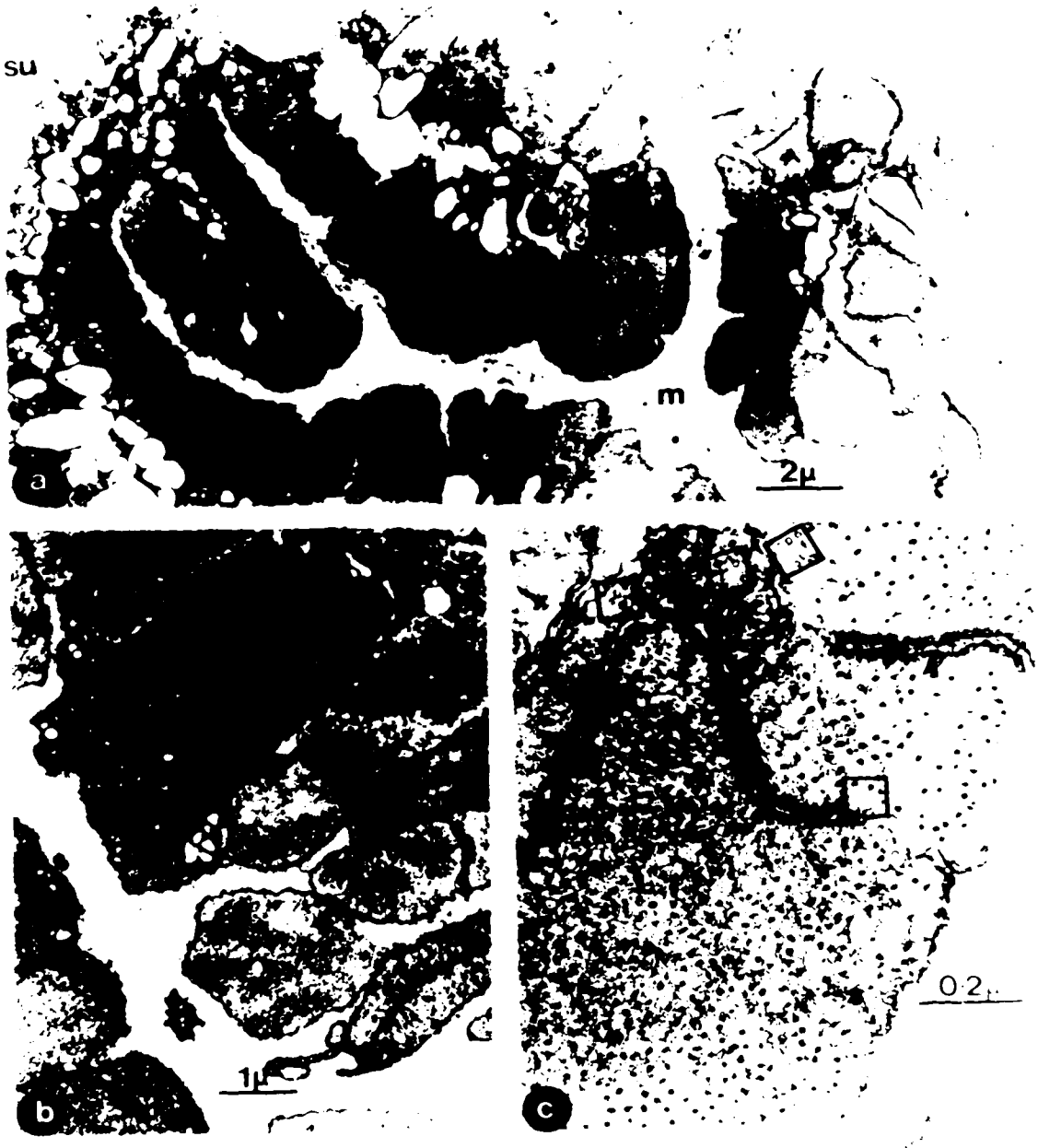

su
m
a
2μ
b
1μ
c
0·2μ

PLATE 11 Connections between radial muscle and radial canal.

a) Transverse section through the perradius showing an epithelial connection (arrow) between ectodermal radial muscle process and the endodermal radial canal. Note the presence of a neurite within the mesoglea midway between radial muscle and radial canal. X4000.

b) Enlargement of the connection between radial muscle and radial canal of a). Myofibrils (arrowheads) are present within the epithelial connection. X29,000.

c) Another transverse section of the same epithelial connection in a) showing a more extensive union (arrow) between radial muscle and radial canal. The presence of radial muscle processes associated with a neurite within the mesoglea suggests that there is also a nervous connection between radial muscle and radial canal perhaps joining radial nerves and the peripheral canal nerves. X5000.

Symbols: m - mesoglea, n - neurite, rc - radial canal, rm - radial muscle.

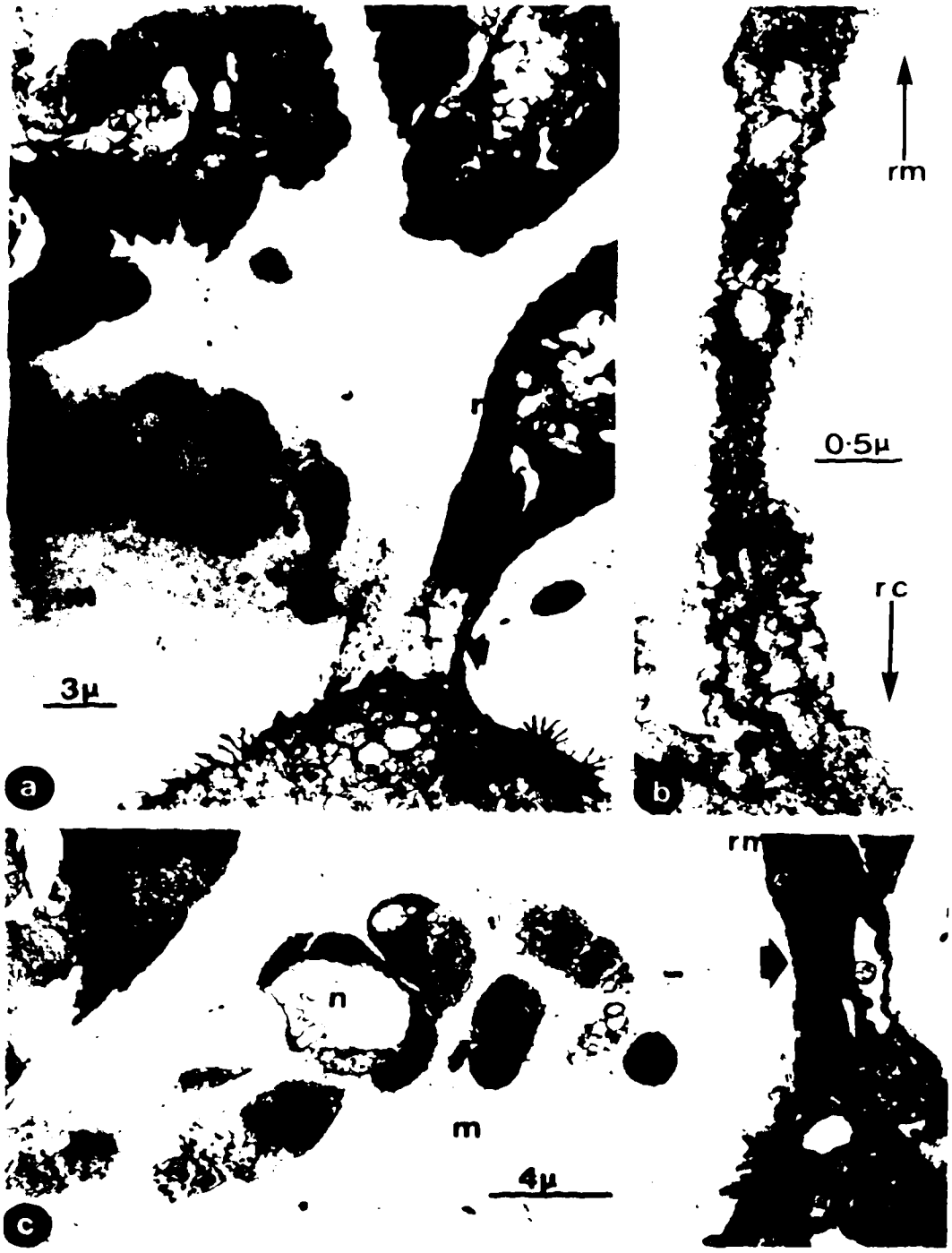

3μ
a
rm
0·5μ
rc
b
n
m
4μ
c
rm

PLATE 12 General ultrastructure of radial nerve.

a) Radial section showing the apposition of the radial nerve bundle (rn) with radial muscle (rm) and swimming muscle (sm). A neurite (n) distal from the radial nerve bundle can be seen near the mesoglea (m). The arrowhead indicates a synapse of radial nerve with a swimming myoepithelial cell (see plate 13a). X5000.

b) Gap-junction (gj) between radial neurites and an annular gap-junction (agj) in which the entire perimeter of a neuronal process is tightly apposed to another neurite. X50,000.

c) d) Asymmetrical synapses between radial neurites with pre- and postsynaptic membrane densification and presynaptic vesicles with dense (c) and clear (d) cores. c) X28,000. c) X27,000.

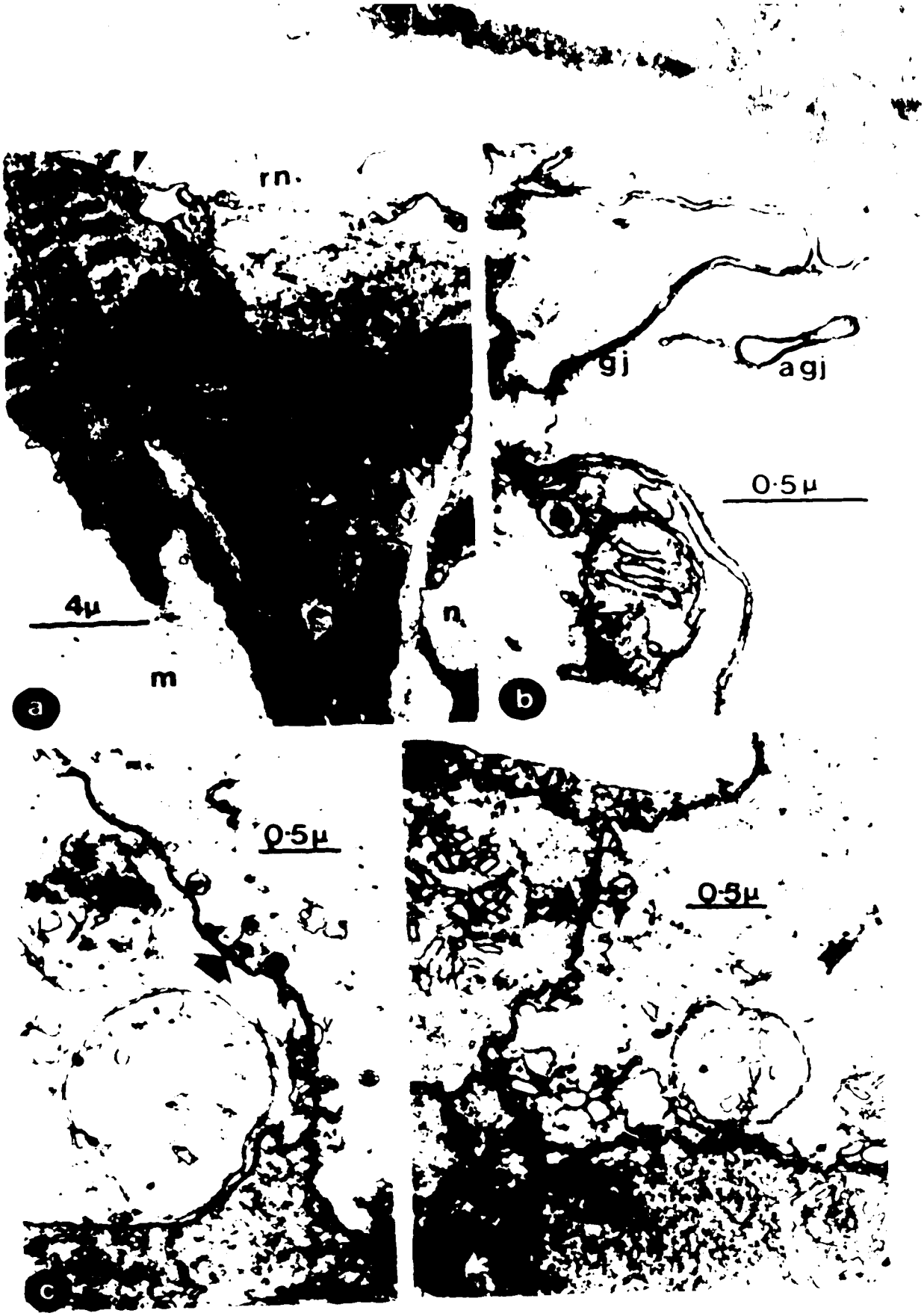

rn.
4μ
m
n
a
gj
agj
0·5μ
b
0·5μ
0·5μ
c

PLATE 13 Synapses between radial nerve and swimming muscle.

Synapses (arrows) between radial neurites (rn) and apical extensions of the striated, circular swimming myoepithelium (sm). These neuro-myoepithelial junctions are characterized by pre- and postsynaptic membrane densification, an intercellular space of about 25nm and usually clear-cored presynaptic vesicles of variable diameter between about 85-200nm. Microtubules (mt) cut in longitudinal section can be seen at the apical end of the swimming myoepithelial cells in a). a) X24,000, inset X55,000; b) X15,000, inset X46,000.


m
0·2μ
0·5μ
cm

PLATE 14 Synapses between radial nerve and radial muscle.

Four synapses (arrowheads) between radial neurites (rn) and basal processes of smooth, radial myoepithelial cells (rm). These junctions are more common than radial nerve-swimming muscle synapses (plate 13). Microtubules (mt) can be seen in the radial myoepithelium of a). Characteristics of these neuro-myoepithelial junctions are pre- and postsynaptic membrane densification, an intercellular space of about 10nm and presynaptic vesicles, either clear-cored (a, d) or dense-cored (b,c). of 60-100nm. a), X36,000; b) X9000, inset X117,000; c) d) X37,000. e) X89,000.

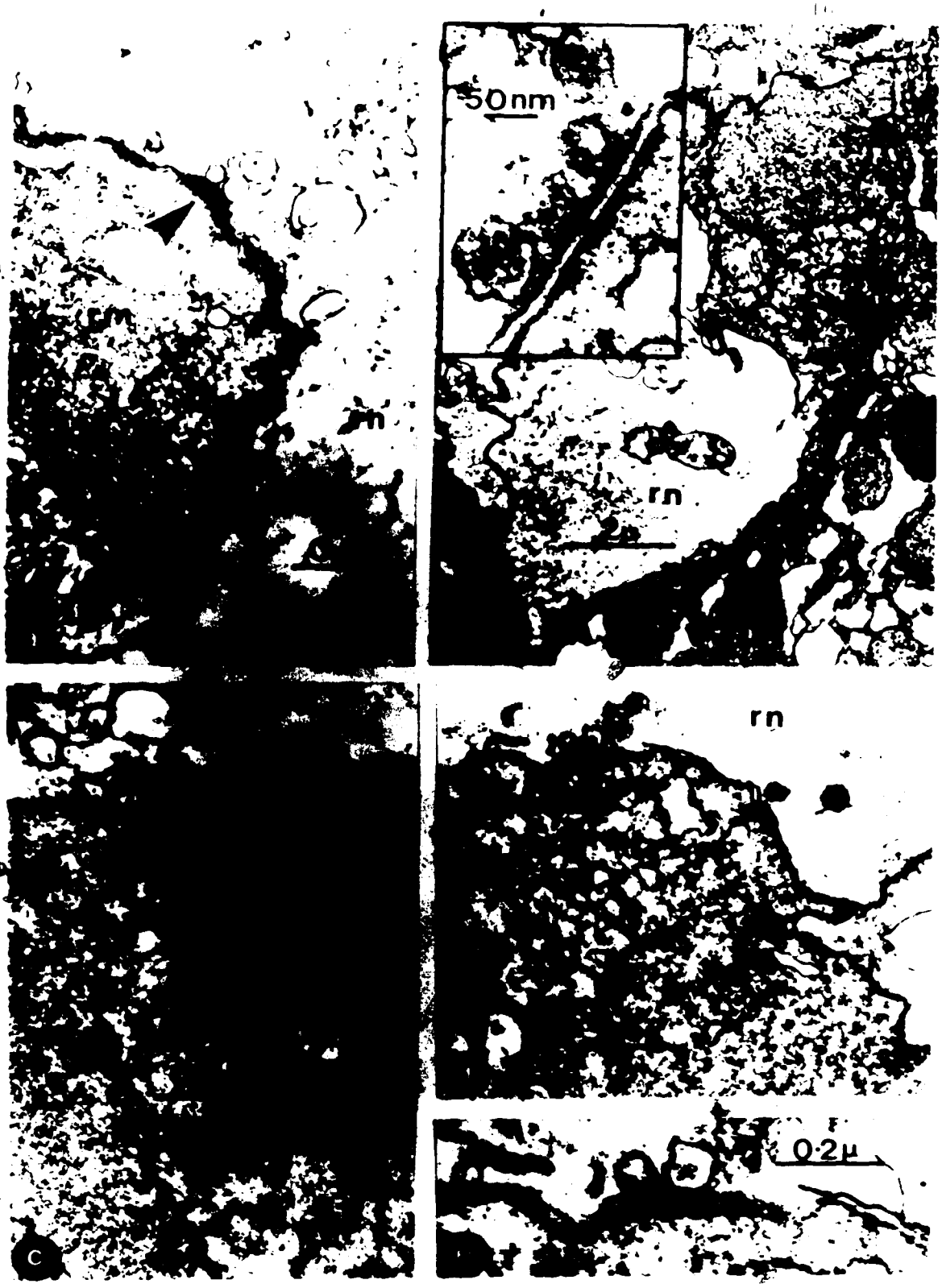

50nm
rn
rn
0·2μ
C

PLATE 15 General ultrastructure of sphincter muscle and outer nerve-ring.

a) Radial section through interradial margin at the triradius (t) where mesoglea (m) of the exumbrella (to the right), subumbrella (to the left) and velum (above) converge. Sphincter muscle (sp m), across from the endodermal ring canal (rnc), extends to the triradius and passes a short distance up the velar mesogleal face, in close apposition to the outer nerve-ring (onr). X3000.

b) Radially sectioned sphincter muscle showing the following similarities to radial muscle: mesogleal (m) infoldings, proximity to endoderm (the ring canal; rnc), "desmosome-like" junctions (arrowheads), microtubules (boxes). X12,000.

c) Three asymmetrical synapses (arrowheads) with dense-cored vesicles between outer nerve-ring neurites. X41,000.

d) A symmetrical synapse (arrowhead) between outer nerve-ring neurites with dense- and clear-cored vesicles. In this case, if the contact is functional both neurons must have pre- and postsynaptic capabilities. X26,000.

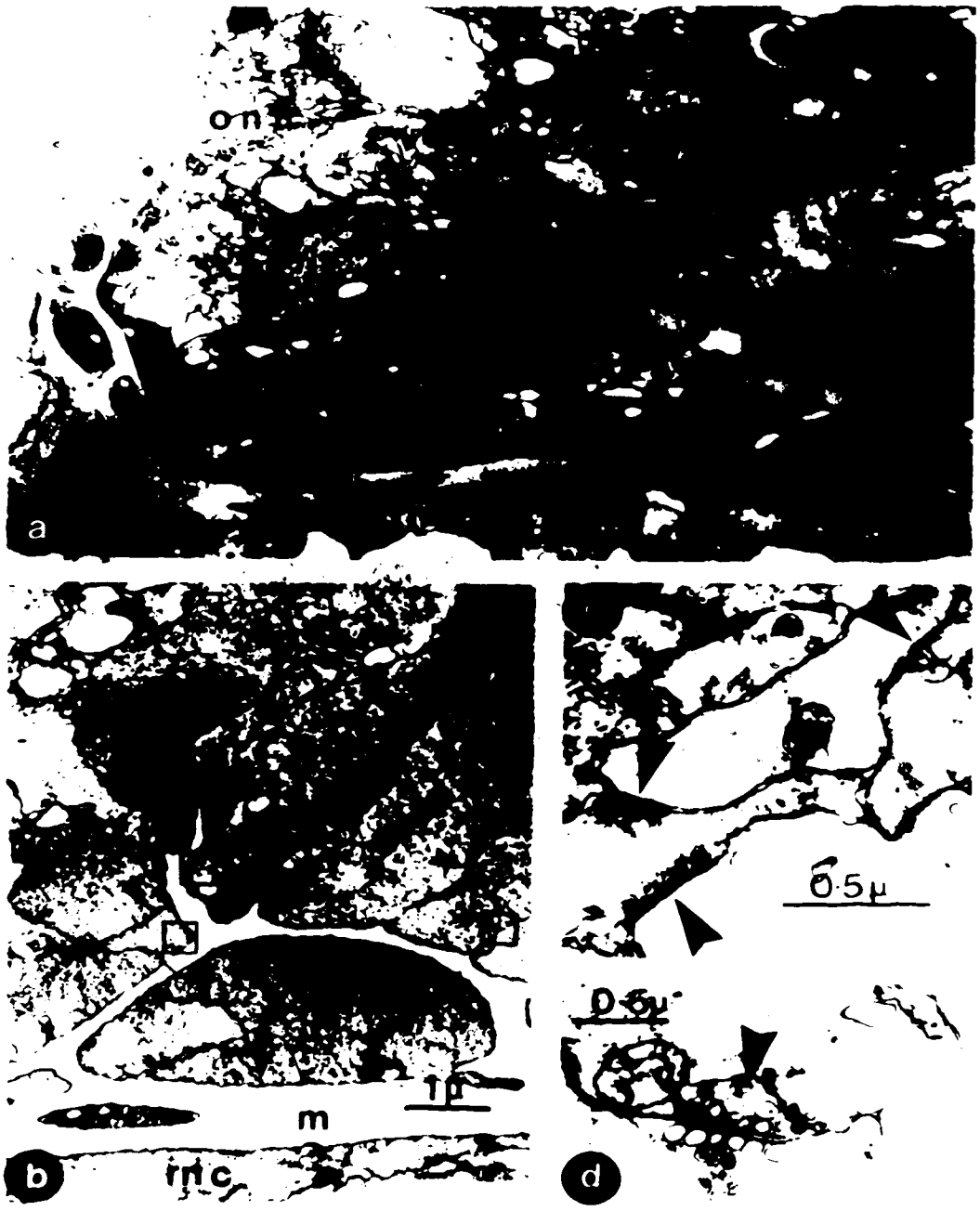

on
a
b
m
1μ
rnc
0·5μ
0·5μ
d

PLATE 16 Synapses between outer nerve-ring (ONR) neurites and sphincter muscle.

Radial sections of synapses (arrowheads) between ONR neurites and processes of smooth, circular, sphincter myoepithelium near the triradius. These junctions are found at the face of mesoglea passing to the velum (up in a and b; down in c-e).

Symbols: inr - inner nerve-ring, m - mesoglea, n - neurite from ONR, onr - outer (exumbrellar) nerve-ring, rnc - ring ca[illegible], spm - sphincter muscle, t - triradius (converge[illegible] exumbrellar, subumbrellar and velar mesoglea). a) X4000, b) [illegible] X4000, d) X9000, [illegible] X39,000.

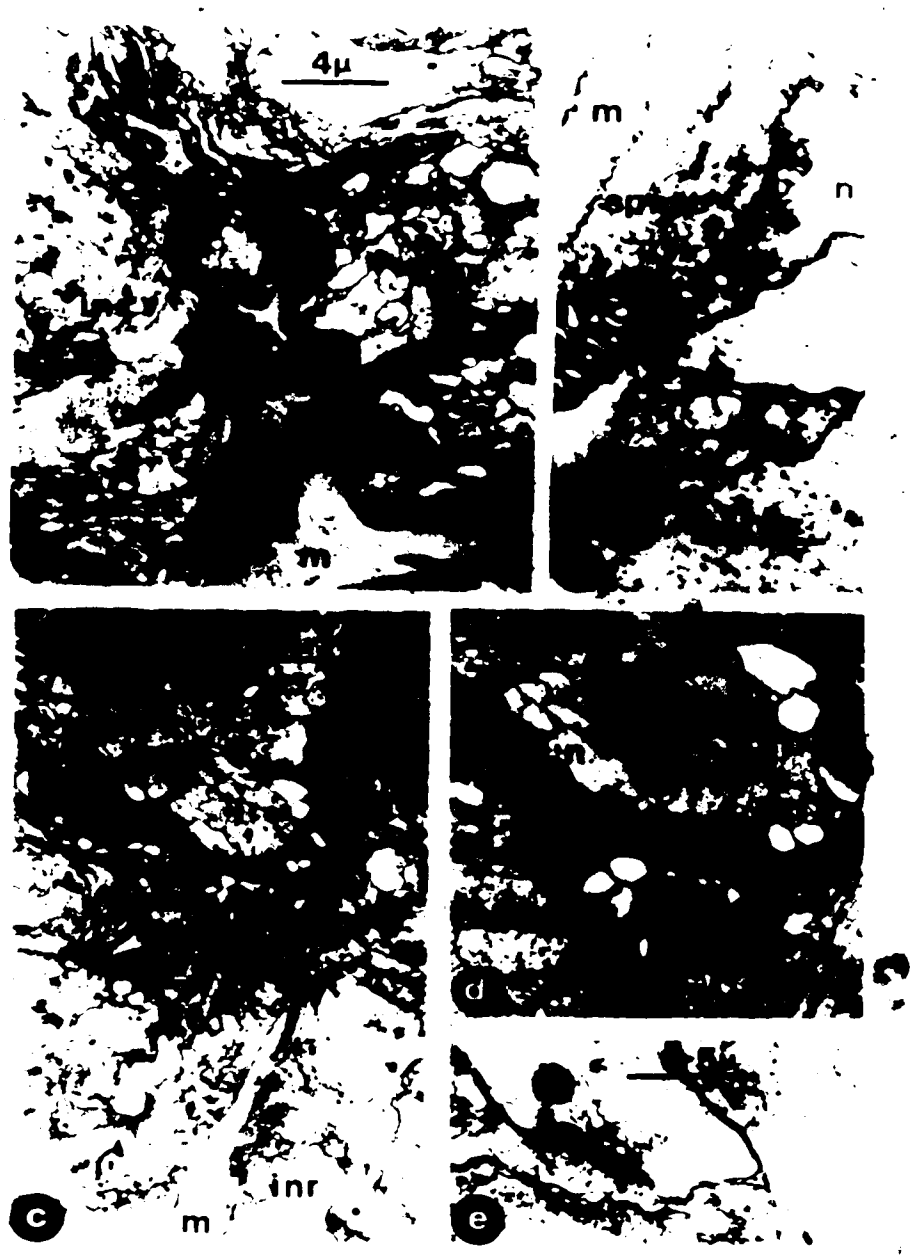

4μ
m
m
n
c
m
inr
d
e

PLATE 17 General histology of the perradial margin.

Light micrographs of radial sections through the margin at the perradius. In all cases subumbrella is up and exumbrella down. Notable features of this region of the margin are: the apparent absence of the sphincter muscle bundle from the exumbrellar-mesogleal interface (cf. Plate 2a); connections between subumbrella and exumbrella, especially apparent in d), e) and f), involving muscular and nervous elements; and connections between radial muscle processes and ring canal, arrowed in b) and d) inset.

Symbols: em - muscle within exumbrella possibly originating from the subumbrella, inr - inner nerve-ring, m - transmesogleal muscular process, n - transmesogleal nervous process, onr - outer nerve-ring, rm - radial muscle, rnc - ring canal, t - triradius (convergence of exumbrellar, subumbrellar and velar mesoglea), v - velum. a) X1000, d) X2000; b), c), d) inset, e) and f) X3000.

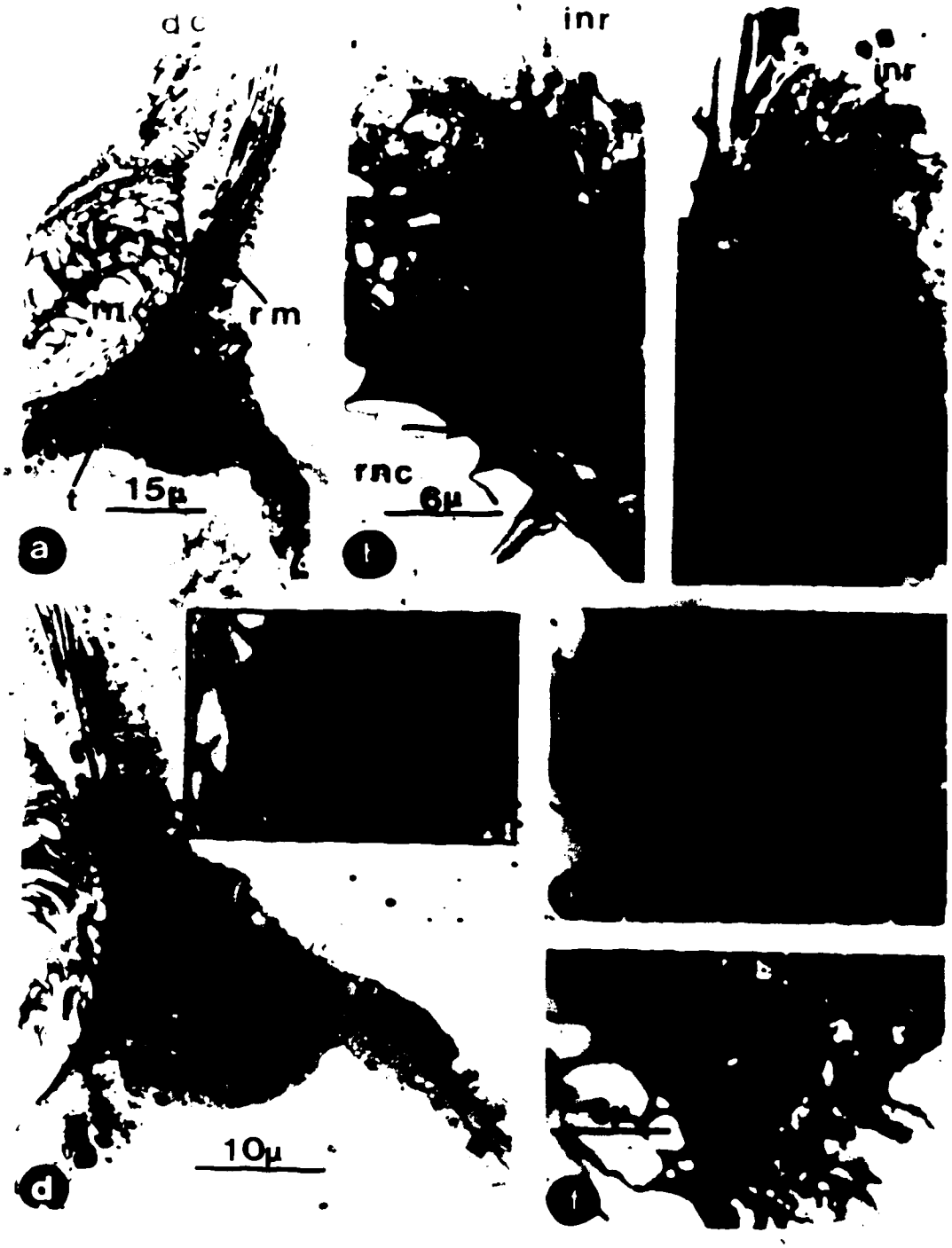

dc
inr
inr
rm
rm
t
15µ
rnc
6µ
a
f
10µ
d
f

PLATE 18 Ultrastructure of perradial margin.

Radial section through the margin at the perradius with subumbrellar side down and exumbrellar side up. Sphincter muscle is greatly reduced along the exumbrellar-mesogleal interface (cf. Plate 15a). Muscle on the exumbrellar side of the margin involves long strands of smooth muscle processes (arrowheads) passing through the outer nerve-ring almost to the exumbrellar surface, possibly originating from the subumbrellar radial muscle. Boxes indicate the location of synapses between ONR neurites and exumbrellar smooth muscle shown at higher magnifications in Plate 21.

Symbols: em - exumbrellar (sphincter and/or radial) muscle, exs - exumbrellar surface, inr - inner nerve-ring, m - mesolgea, onr - outer nerve-ring, rm - radial muscle, rnc - ring canal, t - triradius (convergence of subumbrellar, exumbrellar and velar mesoglea). X2000.


rnc
t
i

PLATE 19 Ultrastructure of subumbrellar-exumbrellar connections at the perradial margin.

Radial sections through perradial margin at the triradius with subumbrella to the right, exumbrella to the left and velum up. Transmesogleal connections from subumbrella to exumbrella include both muscular (arrowheads) and nervous elements. This suggests that the exumbrellar muscular processes, passing through the outer nerve-ring towards the exumbrellar surface and along the velar mesoglea interface (see Plate 18), are continuous with the radial muscle.

Symbols: inr - inner nerve-ring, m - exumbrellar muscle, n - transmesogleal nervous connection, onr - outer nerve-ring, rm - radial muscle, rnc - ring canal, t - triradius (convergence of exumbrellar, subumbrellar and velar mesoglea). a) X2000, b) X5000, c) X3000.

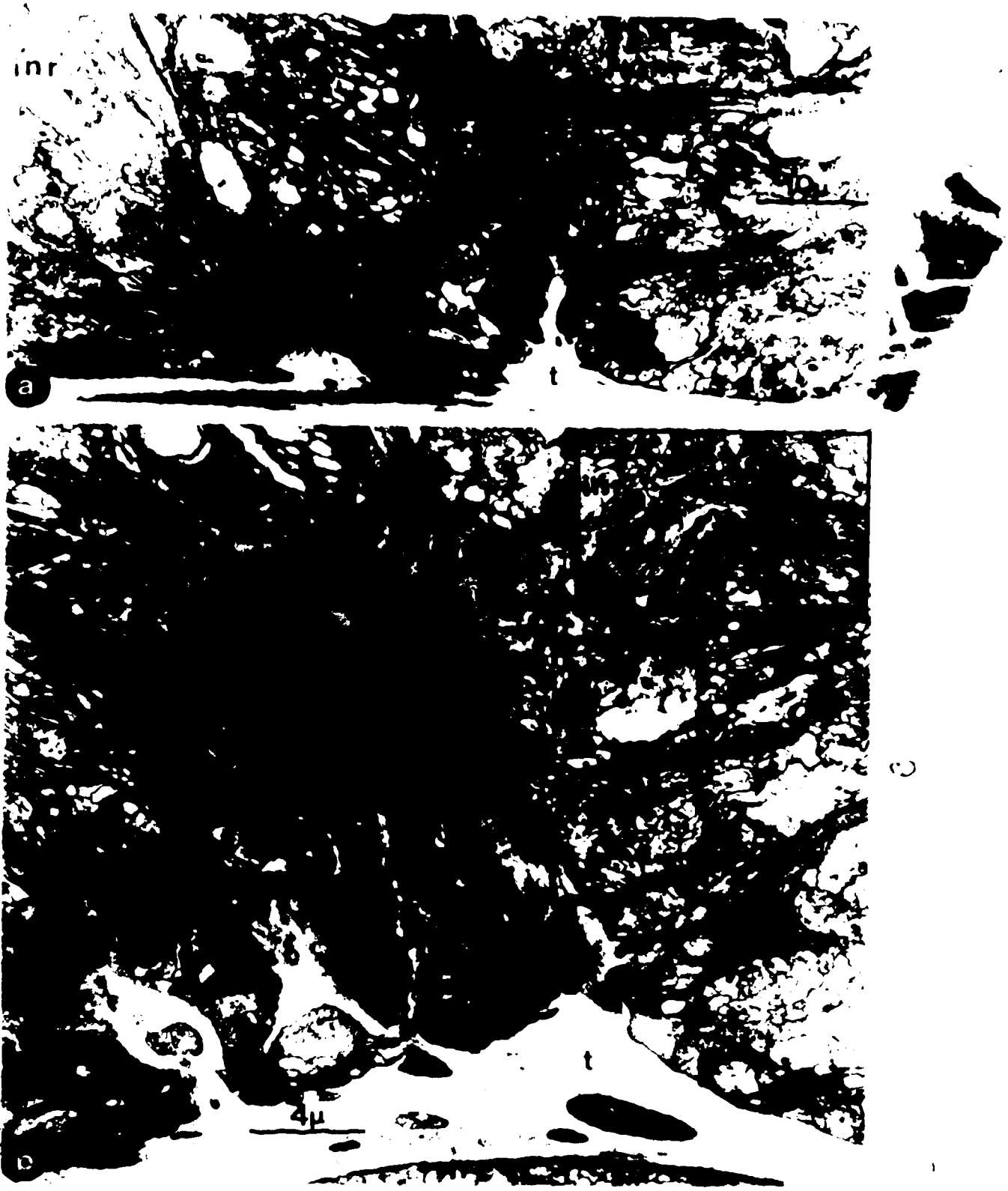

inr
a
t
b
t
4μ

PLATE 20 Synapse of inner nerve-ring (INR) neurite with radial muscle.

Radial sections through the perradial margin a short distance from the triradius (below) with subumbrella to the left and exumbrella to the right. Transmesogleal radial muscle processes (arrowheads) continuous with exumbrellar muscle are seen in a). Box indicates a synapse between an INR neurite and radial muscle (enlarged in b and c). This synapse has an intercellular space of about 10nm, a large number of fairly homogeneous presynaptic vesicles with diameters of about 60nm and with electron dense cores, and a peculiar tubular vesicle continuous with the intercellular space (arrowhead in c).

Symbols: inr - inner nerve-ring, m - exumbrellar muscle, n - neurite from INR, onr - outer nerve-ring, rm - radial muscle.

a) X5000, b) X14,000, c) X80,000.

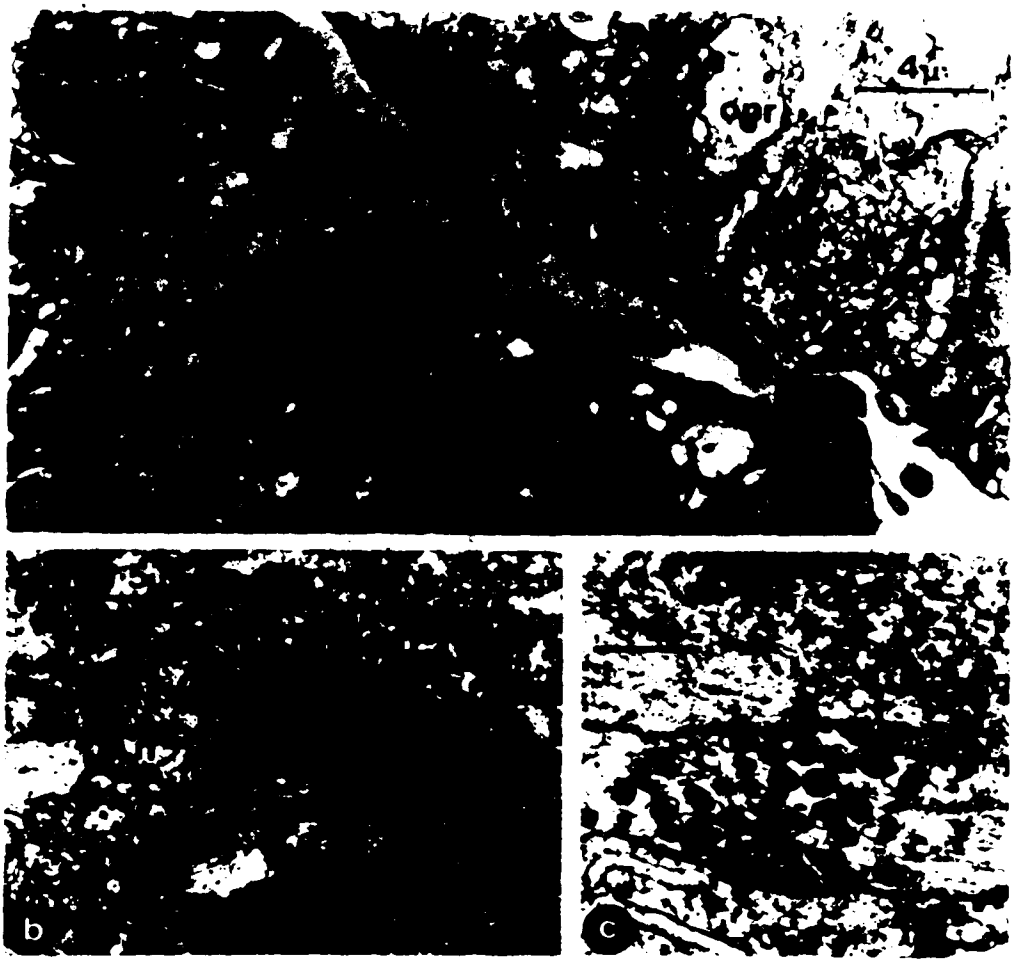
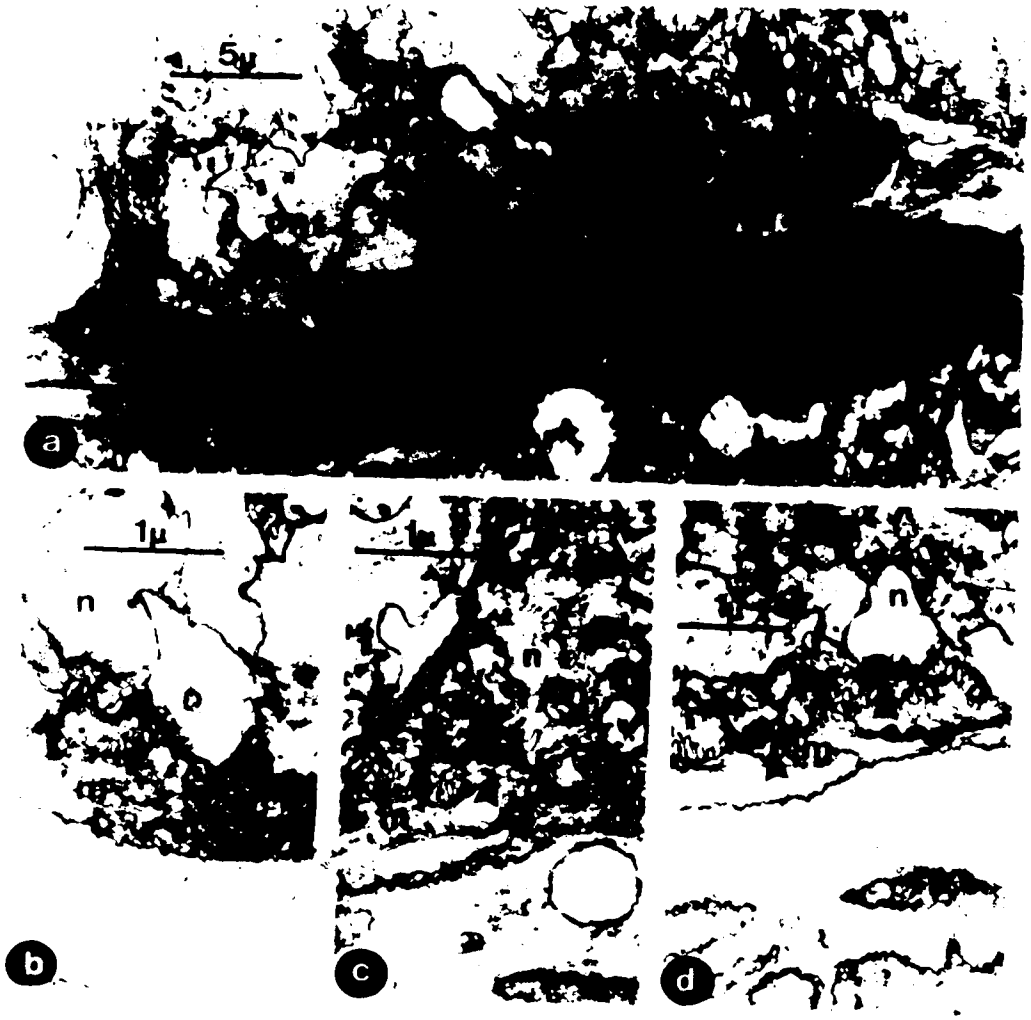

4μ
opr
b
c

PLATE 21 Synapses between outer nerve-ring (ONR) neurites and smooth muscle at the perradius.

Radial sections of perradial margin near the triradius (to the right) with subumbrella below and exumbrella above. Three synapses (boxes) between ONR neurites and exumbrellar smooth muscle at the velar mesogleal face (for exact location at the margin see Plate 18). These neuro-myoepithelial junctions (arrowheads) are enlarged in b), c) and d) showing the usual pre- and postsynaptic membrane densification and presynaptic vesicles.

Symbols: inr - inner nerve-ring, m - exumbrellar smooth muscle, n - ONR neurite, onr - outer nerve-ring, rm - radial muscle.

a) X4000, b) X21,000, c) X19,000, d) X17,000.


5μ
a
1μ
n
b
1μ
n
c
n
d